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Combining and mapping QTL for fusarium head blight (FHB) resistance in wheat

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COMBINING AND MAPPING QTL FOR FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN WHEAT

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For my grandparents

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ABSTRACT

Sun, Jin. Ph.D., Purdue University, December 2014. Combining and Mapping QTL for Fusarium Head Blight (FHB) Resistance in Wheat. Major Professor: Christie E. Williams.

Fusarium head blight (FHB) has become one of the most damaging wheat diseases in humid and semi-humid regions around the world. Single gene resistance to FHB in wheat provides only partial resistance and also the disease severity is highly influenced by environment. Consequently multiple genes are required for effective resistance. Our hypothesis is that identifying DNA markers for type I resistance will be very beneficial for selection, and combining type I and type II FHB resistance will be more effective than either type alone. The objectives of this project are to 1) combine type I resistance from cultivars Goldfield, INW0412, Bess, 99751, and Truman; and type II resistance of *Fhb*1 and *Qfhs.pur-7EL* backcrossed into adapted soft winter wheat lines and quantify augmentation of FHB resistance and 2) characterize a RIL population from the cross INW0412 (type I resistance)/992060G1 (susceptible) for frequency of initial infection and map QTLs for type I resistance. For objective 1, QTL from Sumai3 on chromosome 3B (*Fhb1*), from tall wheatgrass on 7EL (*Qfhs.pur-7EL*), and from Goldfield together provided high resistance, whereas epistatic interactions among those three QTL resulted in lower resistance than expected. QTL from Sumai3 and from tall wheatgrass on 7EL (*Qfhs.pur-7EL*) each significantly improved type II FHB resistance.

No effect on increasing type I FHB resistance was detected in the presence of the QTL on 2B in these lines, which may be overshadowed by other potential genes controlling type I resistance that presented. Combining cultivars with type I and type II FHB resistance provided lines with high FHB resistance that will be beneficial to improve wheat cultivars. For objective 2, a population of 198 RILs and the two parents were characterized for FHB incidence at Lafayette, IN in 2011 and 2013 and in the greenhouse 2012 and 2013. A two-enzyme genotyping-by-sequencing (GBS) approach was applied to construct a 1,883 cM linkage map. Composite interval mapping analysis detected a QTL on chromosome 1AS under greenhouse conditions, and three other QTL on chromosomes 1BL, 2BL, and 3AS under field environments. Each QTL explained between 7.44% and 12.20% of the total phenotypic variation. RILs with all three QTL on chromosomes 1BL, 2BL, and 3AS significantly improved type I resistance by 33.06% in the field experiments. Our results also confirmed that type I and type II FHB resistance were controlled by different loci in wheat.

CHAPTER 1. INTRODUCTION

1.1 Fusarium head blight

Fusarium head blight (FHB), also commonly known as scab, is one of the most damaging wheat (*Triticum aestivum* L.) diseases in humid and semi-humid regions around the world. This fungal disease has been reported as a threat to wheat yield, causing economic losses due to mycotoxin accumulation in many countries, including United States (Nganje, 2004), Canada (Gilbert and Tekauz, 2000), China (Bai and Shaner, 2004) and Netherlands (Snijders, 1990). In the United States, the total direct and secondary economic losses caused by FHB in wheat and barley were estimated to be about $7.7 billion from 1993 to 2001(Nganje et al. 2004). During this 8-year period, losses were especially severe for North Dakota, which suffered close to 45% of the total US losses. In Canada, economic losses for wheat were estimated to be about $220 million in Quebec and Ontario in the 1990s, and $300 million in Manitoba from 1993 to 1998 (Windels 1999). In China, FHB has leaded to more than 1 million tons of yield losses during severe epidemic years (Bai and Shaner, 2004).

FHB is mainly caused by five species - *Fusarium graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* (Parry et al. 1995) - but the predominant causal fungus in North America and many other countries around the world is *F. graminearum*. *F. graminearum* produces mycotoxins, including trichothecene nivalenol

(NIV), trichothecene deoxynivalenol (DON) and zearalenone (ZEN), which are harmful to animals and humans (Boenisch and Schäfer, 2011); humans may suffer nausea, vomiting, fever, and animals could acquire reproductive disorders (Pirgozliev et al. 2003). Thus, it is necessary to limit the mycotoxin concentration for animal feed and human consumption. The European Union limits for DON are 750µg/kg for grain and flour and 500µg/kg for retail food products, whereas the United States set the tolerance level at 1000µg/kg in wheat productions for human consumptions (Egmond et al. 2003). Canada recommended the maximum DON concentration of 1200µg/kg for soft wheat flour in adult food and 600µg/kg in infant food and China's DON limit is 1000µg/kg for wheat and wheat flour (Egmond et al. 2003). All of these limitations are essential and beneficial for animal and human health.

1.2 Infection and symptomes

The fungus *F. graminearum* Schwabe (*Gibberella zeae* Petch) overwinters as saprophytic mycelia on previously infected crop debris (Goswami and Kistler, 2004), corn stalks, wheat stubble or other host plants. When the weather becomes warm and humid in spring, conidia and perithecia are formed and matured to produce ascospores (Goswami and Kistler, 2004; Trail, 2009). Sexually developed ascospores and asexual macroconidia are the major inoculums to crops via aerial dispersal, but chlamydospores and hyphae can also initiate infection (Sutton, 1982; Bai and Shaner, 2004). Wheat heads are most vulnerable to infection at anthesis (Sutton, 1982). Usually, FHB fungi initially colonize extruded anthers of spikelets, and later water-soaked and dark-brown spots become visible on the glumes of infected florets (Bai and Shaner, 2004). For susceptible cultivars, FHB fungi invade from infected floret to adjacent florets within a spikelet in a

horizontal direction, and also invade through vascular bundles of the rachis from infected spikelet to adjacent spikelets within the head in a vertical direction (Goswami and Kistler, 2004; Ribichich et al. 2000). Finally, florets and spikelets become prematurely blighted throughout the entire susceptible wheat head (Schmale and Bergstrom, 2003). It is uncommon that macroconidia and ascospores directly penetrate glumes, palea, or rachis (Bai and Shaner, 2004). As time goes on, the fungus colonizes the developing kernels, leaving them shriveled with pink or light-brown coloration (Schmale and Bergstrom, 2003).

1.3 Genetics of *Fusarium graminearum*

F. graminearum contains four chromosomes, and the 36.1 Mb genome of *F. graminearum* has been sequenced (Cuomo et al. 2007). Researchers observed a small number of repeat sequences in the genome, and speculated that the process of repeat-induced point mutation (RIP) is occurring, which identifies and mutates duplicated sequences by introducing C:G to T:A transition mutations in both copies during the sexual stage of the fungus (Galagan and Selker, 2004). Genome annotation identified 13,718 protein-encoding genes over the four chromosomes (Wong et al. 2010). These studies offer an overall summary of genic information for plant-pathogen interactions, particularly in high diversity regions related to disease infection that cause rapid adaptation of the FHB fungus to various environments or hosts (Cuomo et al., 2007).

1.4 Trichothecene mycotoxins

As secondary metabolites of fungi, mycotoxins accumulate in infected plants, resulting in economic losses and toxicity to humans and animals. Trichothecene are a major class of mycotoxins, with more than 200 toxins in this family sharing the same

core structure: tricyclic 12, 13-epoxytrichothec-9-ene (EPT) (McCormick et al. 2011). Four types (type A, B, C, and D) of trichothecene are distinguished by their different chemical structures (Kimura et al. 2007). Type A, B, and C differ by the substitution at the C-8 position; T-2 toxin (type A), an ester function; DON (type B), a carbonyl function; and crotocin (type C), a C-7/C-8 epoxide (McCormick et al. 2011). Type D trichothecenes contains an ester-linked macrocycle between the C-4 and C-15 positions (Foroud et al. 2009). Although type A and B trichothecenes are associated with FHB, type B is predominant, and includes deoxynivalenol (DON) and its derivatives. Trichothecenes inhibit eukaryotic protein translation, mitosis, and DNA and RNA synthesis. Inhibition of these processes triggers wilting and necrosis in plants, reduces seed germination and growth, and causes reproductive disorders and reduced ovarian functions in animals (Rocha et al. 2005).

1.5 Trichothecene biosynthesis

Fusarium trichothecene biosynthesis starts with the cyclization of farnesyl pyrophosphate (FPP), as a main intermediate in protein isoprenylation, to form a non-toxic trichothecene product, trichiodiene (TDN) (Cardoza et al. 2011; McCormick et al. 2011; Foroud et al. 2009). Oxygenation steps then create oxygenated derivatives of trichiodiene, including isotrichodermol formation as the first trichothecene (isomerization and second cyclization steps), calonectrin (CAL) formation from isotrichodermol, and trichothecene structures produced by an esterification (such as DON or NIV) (Kimura et al. 2007; Cardoza et al. 2011; McCormick et al. 2011).

Many genes involved in trichothecenes biosynthesis are found in a 25kb gene cluster (*Tri*) (Goswami et al. 2004; Kimura et al. 2007; Cardoza et al. 2011). For *F.*

graminearum, 12 *Tri* genes in the cluster are up-regulated during trichothecene biosynthesis (Kimura et al. 2007). *Tri6* relates to a zinc finger transcription factor, and *Tri10* encodes a type of regulatory protein (Kimura et al. 2007). The function of *Tri9* and *Tri14* genes are still unknown, but three of the remaining seven are oxygenase genes: *Tri4* (C-2 oxygenation) producing isotrichotriol, *Tri11* (C-15 oxygenation), and *Tri13* (C-4 oxygenation). Two of the genes are acetyltransferases: *Tri7* (C-4 acetylation) and *Tri3* (C-15 acetylation). *Tri8* (C-3 deacetylation) is an esterase gene and *Tri12* (MFS transporter) is a transporter pump gene. The last gene in this cluster is *Tri5* (trichodiene synthase), which catalyzes the initial substrate to create trichiodiene (Goswami et al. 2004; Cardoza et al. 2011; Alexander et al. 2004). Besides those 12 genes, four others involved in trichothecene biosynthesis are locate outside the *Tri* cluster, including *Tri101* (encoding trichothecene 3-O-acetyltransferase), *Tri1* (P450 oxygenase gene), *Tri16* (acyltransferase), and a newly characterized gene *Tri15* (negative regulator of some trichothecene biosynthetic genes) (Kimura et al. 2007; Alexander et al. 2004).

1.6 Types of resistance

In 1963, Schroeder and Christensen reported two types of resistances to FHB in wheat: resistance to initial infection (type I resistance) and resistance to the spread of disease within the head (type II resistance). Different inoculation and screening approaches are applied to distinguish these two types of FHB resistance. For type I resistance studies, both sides of each spike are spray-inoculated with a suspension containing 12,000-14,000 conidiaspores per ml dH_2O, either early in the morning or in the evening, of the day on which 50% of the florets in the spike are flowering. The percentage of spikes that are diseased (FHB incidence) is determined 14 days after spray

inoculation. Inoculation time, evaluation time, and inoculum concentration are critical to identify type I FHB resistance (Bai and Shaner, 2004). If inoculations are carried out before the anthers are extruded from the florets, susceptible cultivars may easily escape the disease and appear to show type I resistance (Bai and Shaner, 2004). Also if the evaluation time is too late, or inoculum concentration is too high, then different levels of type I resistance are difficult to distinguish among various cultivars, and may be confused with type II resistance (Bai and Shaner, 2004).

For type II resistance screening, point inoculation is used to quantify resistance to spread of the disease. The spikelet is injected with 10ul of a *F. graminearum* suspension consisting of 50,000 conidiaspores per 1ml dH_2O. The site of inoculation is the 3rd or 4th spikelet from the tip when the spike is at 50% anthesis (the day on which 50% of the flowers in the spike are open with extruded anthers). Type II resistance is measured as the percentage of spikelets per spike that are diseased at 20-22 days depending on weather conditions after point inoculation. Type II resistance evaluation is not as sensitive and difficult as type I resistance, and it has been widely identified in various wheat cultivars around the world.

In 1995, Mesterhazy proposed five types of active resistance; in addition to type I and type II FHB resistance, three more types of resistance were summarized. Resistance to DON accumulation is referred to as type III resistance. Miller et al. (1985) demonstrated that resistant wheat cultivars have relatively less DON compared to susceptible wheat cultivars under the same pathogen infection conditions and environment. There are two main reasons for low DON concentration in resistant cultivars: factors that inhibit DON accumulation, and factors that promote DON

degradation. Either or both of them can be operating in resistant cultivars. Miller and Arnison (1986) proved that FHB resistant cultivar ‘Frontana’ degraded more DON than susceptible cultivar ‘Casavant’.

Tolerance, defined as type IV resistance by Mesterhazy (1995), is recognized by no significant differences in yield between control plants and those exhibiting the primary symptoms of FHB. Resistance to kernel infection, measured as the percentage of infected kernels and named type V resistance, is difficult to measure for the reason that type I and II resistance also reduce the level of kernel infection, which is confused to determine the actual level of type V resistance (Shaner, 2002; Bai and Shaner, 2004).

1.7 Mechanism of FHB resistance

Currently, the mechanisms of FHB resistance are still unclear because of their complexity and multiplicity (Xiao et al. 2013). Generally, the mechanisms can be separated into morphological and physiological resistance (Gilsinger et al. 2005). Morphological resistance allows cultivars to escape infection by the fungus, resulting in low incidence of disease, so it can be also called avoidance. Cultivars characteristics such as height, awnedness, and flower opening time during anthesis, contribute to morphological resistance. Hiltona et al. (1999) studied the negative relationship between the resistance of cultivars and the tiller height, two year’s field results showed that taller winter wheat had less FHB symptom severity since taller cultivars are farther away from the crop debris that serves as the inoculum source compared to shorter ones. Mesterhazy (1995) mentioned that cultivars with awns were more susceptible to FHB compared to awnless cultivars under natural infection in the field. And Gilsinger (2005) demonstrated

that narrow flowering time reduced the risk of FHB infection, and identified four SSR markers associated with this trait.

Physiological resistance is usually based upon biochemical pathways that can inhibit the infection by the pathogen (Gilsinger et al. 2005). In order to better understand factors associated with FHB resistance, it is necessary to study the roles of defense-response genes, also called pathogenesis-related (PR) gene (Pritsch et al. 2001). Defense-response genes encode proteins like PR-1 (unknown), PR-2 (β-1, 3-glucanase), PR-3 (chitinase), PR-4 (acidic chitinase), and PR-5 (thaumatin-like protein) (Linthorst and Loon, 1991). Pritsch et al. (2000) have studied the transcripts for defense genes that expressed during *F. graminearum* infection. Except PR-4 and PR-5, other defense response genes were accumulated in both resistant and susceptible cultivars, and induction timing of defense response genes might associated with *F. graminearum* infection (Pritsch et al. 2000). Further observations showed that the direct contact with pathogen is not necessary for the induction of defense response genes in both resistant and susceptible plants (Pritsch et al. 2001). In Li and Yen (2008)'s study, they concluded that the wheat FHB resistance is not associated with any PR genes based on their observations and previous researches. However, in Xiao et al. (2013)'s investigation, PR 5 and PR 14 were critical for FHB resistance controlled by QTL *Fhb1*. Specifically, earlier and more accumulation of PR 5 transcripts was also observed in Pritsch et al. (2000)'s research.

Plenty of researchers found that FHB resistance associated with defense signaling pathways including jasmonic acid (JA), ethylene (ET), salicylic acid (SA), calcium ions, phosphatidic acid (PA), and reactive oxygen species (ROS) (Ding et al. 2011; Gottwald

et al. 2012; Xiao et al. 2013). Although conflict results were attained from different studies, JA signaling was almost investigated in all of those researches. JA and ET are usually involved in plant defense to necrotrophic pathogens, whereas ET regulates plant defense to biotrophic pathogens (Gottwald et al., 2012). However, *F. graminearum* is considered as hemibiotrophic pathogen with a short biotrophic phase before necrotrophic phase (Jansen et al. 2005), the expression of those signaling might be influence by the detection time. In Ding et al. (2011)'s study, SA signaling pathway was activated as early as 6 hours after infection (hai), while JA signaling was activated at 12 hai, and ET signaling was activated between those two time points.

1.8 Environmental influence and management effect on FHB

Environmental conditions are critical to the dispersal of pathogens and the epidemics of FHB. Both temperature and moisture impact the production and distribution of conidia, as well as the infection process. The optimum temperature for the production of ascopores is 15-20℃ (Rossi et al. 2001), production of macroconidia is 32℃ (Xu, 2003), and the highest infection of *F. graminearum* is at 28.0-29.0℃ (Rossi et al. 2001). The *F. graminearum* infection frequency increased with the rise of temperature from 10-30℃, and then went down as temperature increased from 30-35℃, especially after 72 hours incubation (Rossi et al. 2001). The production of ascospores relies on the humidity of the soil, which should remain above 30%, and rainfall is essential for the formation and maturation of perithecia, which are important in production of primary inoculum (Xu, 2003). Thus, understanding environmental the influences on FHB infection is beneficial for us to estimate the disease risks and to develop valuable disease management approaches. Disease forecasting models are established for farmers and

breeders based on long-term field observations. Those models generally combine the effects of temperature and moisture over the variable of time to predict the FHB risks, and the estimated results depend on the factors used in the model (Xu, 2003). It is still essential and challenging to create a model that can be stable and widely applied.

Field management strategies, such as soil tillage and crop rotation, also considerably influence the level of FHB infection and quality of crops. Soil tillage can affect the location and amount of previous crop residues, such as wheat straws and corn stalks, which are natural materials for the pathogen to colonize for overwintering. Dill-Macky and Jone (2000) identified that FHB incidence and severity were the highest if wheat followed corn and lowest if wheat followed soybean. Reduced tillage practices following the infected wheat or corn residues in the field would significantly increase the disease severity and DON accumulation on wheat (Koch et al. 2005; Pereyra and Dill-Macky, 2008), however, Koch et al. (2005) pointed that compared to cultivar resistance and crop rotation, soil tillage plays a less important role for FHB infection. Field management strategy alone is not sufficient to control disease, but use of resistant wheat cultivars with application of fungicide is a potent and great choice to reduce DON contamination under high infection conditions (Koch et al. 2005).

1.9 Some disease control strategies

FHB management strategies include various approaches, like chemical, cultural, biological control, and coupled with the application of resistant cultivars (Pirgozliev et al. 2003). However, none of them is effective alone against FHB, whereas combinations of those strategies are reliable to control FHB (Gilbert et al. 2013).

Chemical control of FHB has been widely studied. Paul et al (2008) studied the efficacy of triazole-based fungicide for control of FHB and DON accumulation in wheat, and identified the efficiency of fungicides containing prothioconazole, metconazole, and tebuconazole + prothioconzaole for FHB and DON control. tebuconazole + prothioconazole was the most effective fungicide for FHB index, while the most effective fungicide for DON was metconazole (Paul et al. 2008). Another group of fungicides, strobilurins, are also demonstrated to control FHB disease. Azoxystrobin significantly reduced the FHB and DON accumulation, while it was much less effective than metconazole in Pirgozliev's experiment (2002). The fungicide cannot stop the growth of fungus once the fungus has penetrated to the plant structure, so as a floral infecting disease, the plants are most vulnerable at anthesis (Yoshida et al. 2012). Therefore, the application of fungicide depends on the accurate timing, dosage, and application methods (Ackermann et al. 2013).

Cultural control, including soil tillage and crop rotation, also reduce the FHB infections. As mentioned earlier, removing crop residues and correct crop rotation reduce FHB inoculum sources on the soil. Fertilizer application can also be considered cultural control; however, it is not clear how nitrogen management affects FHB, and the results of different studies are not consistent. Lemmens et al. (2004) concluded that nitrogen fertilizer application doesn't affect FHB management of wheat cultivation based on their observations. But the FHB incidence in spring wheat was reduced if adequate starter nitrogen was applied with early planting based on Subedi's studies (2007).

Biological control (biocontrol) generally involves two strategies, biochemicals and microbial agents, and several reports showed that biocontrol is feasible and has

potential for FHB control (Pirgozliev et al. 2003). Bujold et al (2001) showed that the application of *Microsphaeropsis* sp. P130A isolate significantly reduced the production of ascospores in wheat and corn residue, and also significantly reduced perithecia numbers. Khan et al. (2005) demonstrated that when the soil was mixed with culture filtrate of two bacteria, *Pseudomonas* sp. strain MKB 158 and *P. fluorescens* strain MKB 249 and, the disease level after inoculation by *F. culmorum* on stem base tissue was 31% less compared to the same situation but soil mixed with culture medium. However, biocontrol is restricted by the environmental conditions, shelf life of the micro-organism culture, and compatibility with field practices, so more work is needed to optimize the efficiency (Gilbert et al. 2004).

1.10 FHB resistance sources

Utilizing resistant cultivars is more effective and economic in FHB and mycotoxin control compared to other management strategies (Gilbert et al. 2000). Currently used germplasm for FHB resistance can be separated into three groups based on regions of origin and wheat types (Gilbert et al. 2000; Bai et al. 2004). The first group includes spring wheat from Asia, including Chinese cultivar ‘Sumai 3’ and its ‘Ning’ derivatives, ‘Wangshuibai’ (Lin et al. 2004; Lin et al. 2006), plus Japanese cultivar ‘Nobeoka Bozu’ (Mesterhazy 1995), ‘Shinchunaga’ (Bai et al. 2001) and ‘Nyu Bai’ (Liu et al. 2003). All of those materials, especially for Sumai 3 and its Ning derivatives, have been widely utilized in wheat breeding programs. The second group includes Braziliam spring wheat cultivars ‘Frontana’ (Steiner et al. 2004) and ‘Encruzilhada’ (Bai et al. 2004). The third group includes winter wheat cultivars ‘Praag8’ and ‘Novokrumka’ (Snijders 1990). In addition to those three categories, the United States cultivars, such as

‘Ernie’ (McKendry et al. 1995), ‘Truman’ (McKendry et al. 2005), and ‘Goldfield’ (Gilsinger et al. 2005), are moderately resistant to FHB and also have been utilized in some U.S. breeding programs.

Since the sources of FHB resistance are limited, alien chromosome introgressions are another good choice to augment the resistance level and to broaden the genetic base (Cai et al. 2005; Zeng et al. 2013). The alien chromosomal fragments with the resistant genes, but without obvious linkage drag, are transferred into adapted wheat via translocations (Cai et al. 2005). This strategy has been utilized in various studies. Several substitution and translocation wheat lines were created from alien chromatin E (or the el_2 genome) of wheatgrass *Lophopyrum elongatum* (EE) that contained FHB resistance. In this material, a FHB resistance QTL, *Qfhs.pur-7EL* located in the long arm of $7el_2$, accounts for FHB resistance (Shen et al. 2004; Shen et al. 2007); FHB resistance were reported from the wheat-*Leymus racemosus* introgression lines, and one FHB resistance gene *Fhb3* was found in the short arm of the chromosome 7Lr#1 (Qi et al. 2008); The FHB resistance was also demonstrated by Zeng et al. (2013) in wheat-*Elymus repens* introgression lines, and FHB infection rates varied in eight introgression lines with different genomic constitutions and types of translocations. Two main concerns are existed for alien introgressions: linkage drag of alien chromatin and epistatic effects of alien resistance genes (Cai et al. 2005). Therefore it is critical to select suitable alien chromatin and recipient genotypes (Cai et al. 2008).

1.11 QTL mapping for FHB resistance

Quantitative trait locus (QTL) mapping has been widely studied. A major QTL, *Fhb1* on 3BS, for FHB resistance was mapped from the population of Sumai3 and ‘Stoa’

by Waldron et al. in 1999, later verified by several studies (Anderson et al. 2001; Zhou et al. 2002), and further fine mapped in two populations within a 1.27 cM interval and 6.05 cM interval (Cuthbert et al. 2006). This QTL was found in several other resistance sources from China as well, such as Ning7840 (Bai et al. 1999. Zhou et al. 2002), Huapei57-2 (Bourdoncle et al. 2003) and Ning894037 (Shen et al. 2003), and presently is considered to have the largest effect on type II FHB resistance (Bai et al. 2004). In addition to QTL on chromosome 3BS, type II FHB resistance QTL on chromosomes 5AS (Buerstmayr et al. 2002; Somers et al. 2003) and 6BS (Anderson et al. 2001; Yang et al. 2003) were also repeatedly identified in many researches (Cuthbert et al. 2007). A QTL on chromosome 6BS was later named *Fhb2*, and is flanked by two SSR markers GWM133 and GWM644 (Cuthbert et al. 2007). Other QTL were also identified in those Chinese landraces. Zhou et al (2002) detected two other small QTL on chromosomes 2BL and 2AS in addition to the major QTL on 3BS in the recombinant inbred lines (RIL) from the parents of Ning7840 and Clark. Additional QTL were also identified on chromosomes 2D and 6B in Ning894037 and 'Alondra' RIL (Shen et al. 2003) and on chromosomes 3A and 5B in Huapei57-2 and 'Patterson' RIL (Bourdoncle et al. 2003).

Another Chinese cultivar, Wangshuibai, which has great FHB resistance and unknown relationship with Sumai3, is expected to have a novel QTL other than *Fhb1* (Buerstmayr et al. 2009). Several studies using this cultivar detected QTL associated with type I and type II scab resistance, plus DON accumulation. All of those studies for Wangshuibai detecting QTL on chromosome 3B showed large effects for type II FHB resistance (Ma et al. 2006; Zhou et al. 2004; Jia et al. 2005; Lin et al. 2004; Mardi et al. 2005). This QTL is located in a similar region as the major QTL from Sumai3; both of

them were mapped on the distal end of chromosome 3BS. However, the QTL from Wangshuibai accounts for less phenotypic variance (Ma et al. 2006). Sumai3 was originally from a cross of 'Taiwan wheat' and 'Funo', while Wangshuibai was selected by farmers before the release of Sumai3. There is no exact evidence for the relationship of Sumai3 and Wangshuibai (Zhou et al. 2004). However, Liu et al. (2003) showed polymorphism around this region via Simple Sequence Repeat (SSR) markers. Hence more studies are necessary to identify whether they are different alleles or separate genes near this region (Zhou et al. 2004). Other QTL were also identified on chromosomes 2A (Ma et al. 2006), 7AL (Zhou et al. 2004), 1BL (Zhou et al. 2004), 6B (Lin et al. 2004), 5B (Jia et al. 2005) and 2D (Jia et al. 2005; Mardi et al. 2005) for type II resistance, whereas QTL on chromosome 5A were identified for DON accumulation (Ma et al. 2006), and on chromosomes 4B, 5A, 5B for type I FHB resistance (Lin et al. 2006).

Stable QTL were also detected in other wheat landraces. Brazilian spring wheat Frontana is a popular FHB resistance source. In a Frontana and 'Remus' DH population, a major QTL was mapped on chromosome 3A over three years, and other QTL with smaller effects were also identified (Steiner et al. 2004). Three stable QTL were detected from European winter wheat 'Renan'; one was mapped to chromosome 2B, and the other two were mapped to chromosome 5A (Gervais et al. 2003). Three major QTL were also found in the RIL population of Swiss winter wheat 'Arina' and 'Forno', they were mapped to 6DL, 5BL, and 4AL (Paillard et al. 2004). Winter wheat 'Ernie' is used in U.S. breeding, and QTL that are different from Sumai3 were identified on several chromosomes 2B, 3B, 4BL, and 5A. It is beneficial for breeders to increase cultivar resistance level by stacking those QTL (Liu et al. 2007). Therefore, expanding the QTL

resistance sources is necessary in order for breeders to increase FHB resistance levels during cultivar development. Gilbert et al (2013) mentioned that previously most of studies focused on the QTL of spring wheat in the literature, European and North American breeders started to broaden the materials to more native winter wheat materials based on the introgression of Chinese landraces (Gilbert et al. 2013).

Buerstmayr et al (2009) reviewed the position of reported QTL for FHB resistance, QTL validation, and MAS germplasm from 52 studies, detailed information can be found in that paper (Buerstmayr et al. 2009). Table 1.1 added more reported novel QTL with large effects after 2009.

1.12 Wheat breeding for FHB resistance

The goal of plant breeding is to improve the useful traits based on genetic variation and selection (Asins 2002). By using traditional breeding strategies, mainly based on repeated tests, breeders have improved FHB resistance under natural and artificial epidemic environments (Buerstmayr et al. 2009). However, as a quantitative trait, FHB resistance is complex, and breeding for FHB resistance is further inhibited by the genetic factors of the host and of the pathogen, genotype by environment interaction, undesirable agronomic traits, and phenotyping difficulties (Buerstmayr et al. 2009; Rudd et al. 2001).

Marker-assisted selection (MAS) has been applied to improve the traditional breeding. But Miedaner et al. (2006) mentioned that MAS combined with phenotypic selection would be the best approach to utilize the quantitative variation of disease resistance. Gene pyramiding and introgression, as traditional breeding approaches, usually introduce genes from different gene pools into adapted lines to augment the

resistance. Applying molecular markers could assist in identifying the exact genes integrated into the breeding lines, while reducing the cycle length of selection processes and reducing labor and cost with the improvement of technologies (Miedaner et al. 2006; Shen et al. 2003). For example, two QTL for type I resistance (3B and 5A) and one QTL for type II resistance (3A) from CM82036 and Frontana were pyramided and were introgressed into elite European spring wheat with the help of molecular markers (Miedaner et al. 2006). The stacked donor QTL from 3B and 5A had the highest effect; DON content and FHB disease rate were significantly reduced compared to the susceptible QTL class (Miedaner et al. 2006).

With the rapid development of next-generation sequencing, genotyping-by-sequencing (GBS) that doesn't require preliminary sequence information (Deschamps et al. 2012) has potential benefits for plant breeding. It is valuable for species with large and complex genome sequence information and limited public resources, especially for wheat genetics studies which are restricted by large genome size (17 gigabase pairs) and high repeats (around 80%) (Brenchley et al. 2012). Poland et al (2012) already utilized GBS to develop a high density genetic map with 20,000 SNPs in wheat. Other studies in wheat for FHB resistance using GBS are in progress (information from 2013 National FHB Forum). Genomic selection (GS) is one potential direction for plant breeding, making it possible to increase disease resistance, to identify low impact QTL for disease resistance and to improve the disease resistance germplasm within fewer cycles compared to MAS (Miedaner et al. 2006). Rutkoski et al (2012) confirmed several advantages of GS for

FHB resistance in wheat breeding. However, the applications of GBS and GS are still limited, and more research is needed to develop additional markers and prediction models for FHB resistance.

Table 1.1 FHB resistance with large effects identified after 2009

Location	% of variation	Type of FHB trait	Population	Note	Reference
2A	11.5	Type II	*T. macha* (R[1])/ Furore (MS[3]) BC_2F_3	Georgian spelt wheat *T. macha*, supposed to differ from well-known resistance sources	Buerstmayr et al. 2011
2AL	21-26	Type II	LDN (MS)/LDN(DIC-2A) (S[2]) RICL[4]	Tetraploid wheat cv. Landgdon (LDN) and chromosome 2A substitution line (LDN(DIC-2A))	Garvin et al. 2009
2BL	9.7	Type II	*T. macha* (R)/ Furore (MS) BC_2F_3		Buerstmayr et al. 2011
3A	15	Type II	*T. dicoccum*-161 (R)/Floradur (S) BC_1F_4-derived RIL	Also related to plant height	Buerstmayr et al. 2012
3AS	17.9	Type II	Heyne (MR[5])/Trego (S) RIL	Kansas hard winter wheat	Zhang et al. 2012
3BSc	8.5	Type II	Baishanyuehuang (R)/Jagger (S) RIL	Near centromere	Zhang et al. 2012
4AL	18.1	Type II	Heyne (MR)/Trego (S) RIL	Kansas hard winter wheat	Zhang et al. 2012
4B	7/12.3	Kernel damage	IL94-4653 (R) /Patton (S) RIL	Greenhouse/Field experiment	Bonin et al. 2009
4B	56	Type II	*T. dicoccum*-161(R)/DS-131621(S) BC_1F_4-derived RIL	Co-located with a major height QTL	Buerstmayr et al. 2012
4B	68	Type II	*T. dicoccum*-161 (R)/Floradur (S), *T. dicoccum*-161(R) /Helidur (S) BC_1F_4-derived RIL	Co-located with a major height QTL	Buerstmayr et al. 2012

[1] Resistant; [2] Susceptible; [3] Moderate susceptible; [4] Recombinant inbred chromosome line population; [5] Moderate resistant

Table 1.1 Continued

Location	% of variation	Type of FHB trait	Population	Note	Reference
4DL	13.8-23.4	Type II	Heyne (MR)/Trego (S) RIL	Kansas hard winter wheat	Zhang et al. 2012
5AS	20	Type II	PI277012 (R) /Grandin (S) DH	Also reduce FDK and DON accumulation	Chu et al. 2011
5AL	32	Type II	PI277012 (R) /Grandin (S) DH	Not reported previously, also reduce FDK and DON accumulation	Chu et al. 2011
5AL	23	Type II	*T. macha* (R)/ Furore (MS) BC_2F_3		Buerstmayr et al. 2011
5B	13.8	Seedling	Wuhan-1 (R)/Nyubai (R) DH	Parental information see Somers et al. 2003; and McCartney et al. 2007	Tamburic-Ilincic et al. 2009
6BS	30	Type II	G93010 (R)/ Pelikan (S) RIL	Likely identical to *Fhb2*	Haberle et al. 2009
6B	22.37	Type II	Wangshuibai (R) /Sy95-7 (S) $F_{2:3}$		Zhang et al. 2010
7A	14.62	Type II	Wangshuibai (R) /Sy95-7 (S) $F_{2:3}$		Zhang et al. 2010
7A	22	Type II	CS-Sumai3-7ADSL (R)/ CS[6] (MS) RIL	Designated as *Fhb7AC*	Jayatilake et al. 2011
7A	24	Type III	CS-Sumai3-7ADSL (R)/ CS (MS) RIL	Designated as *Fhb7AC*	Jayatilake et al. 2011
7A	9	Type I	BGRC3487 (R)/2* DT735 (R) BCRIL[7]	Derived from DT735	Ruan et al. 2012
7A	8.6	Type II	BGRC3487 (R)/2* DT735 (R) BCRIL	Derived from DT735	Ruan et al. 2012
7AL	18	Type II	HFZ(R) /Wheaton(S) RIL		Li et al. 2012
7BS/5BL	24	Type II	G93010 (R)/ Pelikan (S) RIL	Overlapped with QTL for plant height and heading date	Haberle et al. 2009
7DL	20.4-22.6/15.9	Type II	Haiyanzhong(HYZ) (R)/Wheaton(S) RIL	Greenhouse/Field experiment	Li et al. 2011

[6] Chinese Spring; [7] Backcross recombinant inbred line

CHAPTER 2. COMBINING QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

2.1 Introduction

Fusarium head blight (FHB), also commonly known as scab, is one of the most damaging wheat (*Triticum aestivum* L.) diseases in humid and semi-humid regions around the world (Bai and Shaner, 2004; Gilbert and Tekauz, 2000; Snijders, 1990). This fungal disease, caused by *Fusarium graminearum*, leads to economic losses due to mycotoxin accumulation in the grain (Nganje, 2004; Gilbert and Tekauz, 2000; Bai and Shaner, 2004). The pathogen produces trichothecene nivalenol (NIV), trichothecene deoxynivalenol (DON) and zearalenone (ZEN), which are harmful to animals and humans (Boenisch et al. 2011).

Mesterhazy (1995) proposed five types of active resistance to FHB; in addition to type I (resistance to initial infection, or low incidence of spike infection) and type II (resistance to the spread of disease within the head) (Schroeder and Christensen 1963), resistance to DON accumulation is referred to as type III resistance, tolerance is designated as type IV resistance, and resistance to kernel infection (percentage of infected kernels) is type V resistance. Utilizing resistant cultivars is an effective and economic approach for FHB and mycotoxin control. Nevertheless, only type II resistance, which is the easiest type of FHB resistance to be assessed under the controlled environments, has been widely studied (Xu et al. 2001; Kolb et al. 2001).

A major QTL for type II FHB resistance, *Fhb1* on chromosome 3BS, was mapped from the cross of 'Sumai3' and 'Stoa' (Waldron et al. 1999) and later verified by Anderson et al. (2001) using the same cross. *Fhb1* was detected from multiple resistance sources from China and is considered the most effective gene for type II FHB resistance with extensive applications (Bai and Shaner, 2004). Alien chromosome introgressions are another potential source for improving type II resistance. Substitution and translocation wheat lines were constructed from a wild wheatgrass (*Lophopyrum*) (Kim et al. 1993), which contains an FHB resistance QTL, *Qfhs.pur-7EL* (or *FhbLoP*, Zhang et al. 2011), located on the long arm of chromosome $7el_2$ and accounts for 15.1 to 32.5% of the phenotypic variation (Shen et al. 2004; Shen et al. 2007). To broaden the sources of resistance for plant breeding, Gilsinger et al. (2005) investigated type I FHB resistance (low incidence) from 'Goldfield' wheat. They identified a major QTL, associated with both narrow flower opening and low FHB incidence, within the region of flanking SSR markers Xbarc200 and Xgwm210 on the short arm of chromosome 2B.

Deployment of single QTL in a wheat variety provides only partial resistance (Shen et al. 2006) because interactions between genotype and environment influence the stability of QTL effects over different environments (Shen et al. 2006; Miedaner et al. 2006). Effective resistance is achieved by pyramiding multiple FHB resistance genes from different sources into adapted lines (Friedt et al. 2007). Applying molecular markers can assist in identifying the exact genes integrated into the breeding lines with pyramided resistance, reducing the cycle length of the selection process, labor and also cost (Miedaner et al. 2006; Shen et al. 2003). The two major QTL, *Qfhs.pur-7EL* and *Fhb1* were successfully stacked and disease resistance was improved (Shen et al. 2006).

Nonetheless, the effectiveness of QTL pyramiding is severely influenced by several issues, including genotype by environment interaction, interaction effects between QTL, and genetic background of the population (Miedaner et al. 2006).

In this study, the frequency of initial infection and FHB severity were characterized in a collection of wheat lines constructed to combine several sources of type I and type II resistance. We tested two hypotheses: 1) combing multiple type I FHB resistance sources would decrease the incidence of initial infection, and 2) pyramiding multiple QTL associated with type I and type II FHB resistance would provide more effective resistance than fewer QTL. Through this process, we produced wheat lines to serve as breeding resources with higher levels of resistance to FHB than the original donor lines.

2.2 Materials and Methods

2.2.1 Plant materials

Goldfield (Ohm et al. 2000) is a Purdue University soft red winter wheat cultivar with type I resistance (low incidence of initial infection) to Fusarium head blight, which is within a 35 cM region between the flanking markers Xgwm210 and Xbarc200 on the short arm of chromosome 2B (Buerstmayr et al. 2009) and associated with narrow flower opening (Gilsinger et al. 2005). For convenience, we will use "*GF"* to represent the QTL on chromosome 2B. Wheat line '99751' ('99751RA1-6-3-94' or 'INW1131') has effective type I resistance (Agricultural Alumni Seed Improvement Association, Inc.; http://ars.usda.gov/Research/docs.htm?docid=21433#inw1131). 'Truman' (McKendry et al. 2005) and 'Bess' (McKendry et al. 2007) both with Type I resistance, are full sibling lines but Bess has earlier maturity. INW0412 has both moderate type I and type II

resistance derived from the Chinese cultivar ‘Huapei 57-2’ (Unpublished data, H. Ohm). The resistance loci in 99751, Truman, Bess and INW0412 are not currently linked to markers. The type II FHB resistance of QTL *Fhb1* came from Sumai3 (Waldron et al. 1999) and is closely linked to STS marker Xumn10 with less than 4 cM genetic distance (Liu et al. 2008; Li et al. 2012; http://hwwgenotyping.ksu.edu/Markers2014.html). Type II resistance of QTL *Qfhs.pur-7EL* is located on the long arm of chromosome $7el_2$ originally from wheatgrass *Lophopyrum elongatum* (also called *Thinopyrum*) (Kim et al. 1993) and is within a 15 cM region between the SSR flanking markers Xcfa2240 and Xbf145935 (Shen et al. 2007). The information for all resistance donor parents is summarized in table 2.1. ‘Patterson’ is susceptible to FHB.

In the 2009 fall greenhouse, multiple crosses were made among cultivars with observed type I FHB resistance, including Goldfield, 99751, Truman, Bess, and. INW0412. In addition, crosses were also made to combine lines containing type I resistance and lines containing type II resistance QTL *Fhb1* and *Qfhs.pur-7EL*. These main lines were also crossed to combinations of various adapted lines from the breeding program that exhibited good performance in a variety of traits. Hence, different F_1 lines were developed from single crosses between two resistance donors. Initially, in the F_1 generation, 203 F_1 seeds harvested from the crosses were planted in plastic trays containing soil (Sunshine Redi-earth professional growing mixes, Sun Gro Horticulture, Agawam, MA) and transferred to the cold room to break dormancy at 2℃ with 12-hour light for three days. Trays were moved to the greenhouse to allow germination for one week and then were vernalized in a cold room at 2℃ and 12-hour light for 65 days. Individual seedlings were transplanted into 0.10 meters plastic pots in the 2010 fall

greenhouse. In the F_2 generation, 1782 F_2 seeds collected from the main head of each F_1 line were planted in the 2011 spring greenhouse. In accordance with our breeding objectives, F_3 seeds to be planted in the field were specifically pre-selected based on the number and types of markers existing in each line based on genotyping results of the parental F_2 generation. Finally, 238 F_2-derived lines (F_3 generation in 2011-12 and F_4 generation in 2012-13) plus four checks (INW0412, 99751, Bess, and Patterson) were seeded in 1m single-row plots, 2 replications at each location, in the fields at Lafayette and Vincennes, IN.

2.2.2 DNA extraction

Total DNA was extracted from two to three cm of young seedling leaf tissue for each plant at both F_1 (203) and F_2 (1782) generation in the greenhouse using the protocol described by Ata-ur-Rehman et al. (2007) and modified by Liu et al. (2013). Tissues were homogenized in DNA extraction buffer composed of 0.1 M Tris-HCl (pH 8.0), 0.05 M EDTA (pH 8.0) and 1.25% SDS. 6 M ammonium acetate was added, and DNA was precipitated in ice-cold isopropanol. DNA was washed with 70% ethanol, suspended in ddH_2O and quantified by a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Samples were adjusted to approximately 80 ng/µl by addition of ddH_2O.

2.2.3 Genetic marker analysis

In 2010, F_1 plants were genotyped to determine the presence or absence of markers linked to type II resistance loci *Fhb1* (Xumn10) and *Qfhs.pur-7EL* (Xcfa2240 and Xbf145935). Then F_2 progeny from marker-screened F_1 plants were tested to confirm marker presence. In addition to markers associated with type II FHB resistance, F_2 plants

were also tested with the markers associated with type I low FHB incidence and narrow flower opening from cultivar Goldfield (Xbarc200 and Xgwm210). For convenience, we will use “type I marker” to represent markers associated with type I FHB resistance and “type II marker” for markers linked to type II FHB resistance. The primer sequences of SSR and STS markers were obtained from published data and the GrainGenes website (http://wheat.pw.usda.gov) (Table 2.2).

PCR amplification was performed in 10 µl volumes including 80 ng of DNA, 1× PCR Buffer #B9014s (New England Biolabs, Ipswich, MA), 0.15mM $MgCl_2$ (Promega, Madison, WI), 0.25µM dNTPs, 0.15µM labeled primer (M13-tailed forward primers were labeled by different fluorescent colors for multiplexing; Applied Biosystems, Foster City, CA), 0.15µM unlabeled primer, and 1 unite Taq DNA polymerase #M0273S (New England Biolabs, Ipswich, MA), using a PTC-100^{TM} MJ Research Thermal Cycler (MJ Research Inc., Waltham, MA). The touchdown PCR program used the following cycling parameters: one cycle of 95℃ for 3 min; ten cycles of 94℃ for 30s, 60℃ for 45s, 72℃ for 1min and decreasing by 1℃ for each of the following ten cycles; thirty cycles of 94℃ for 30s, 52℃ for 45s, 72℃ for 1min; a final extension at 72℃ for 10 min; and short term storage of reaction products at 4℃. The PCR products were prepared for capillary electrophoresis as described by Campbell (2011). The amplified fragments were separated on an ABI 3700 DNA Analyzer (Applied Biosystem, Foster City, CA) at the Purdue University Genomics Center. The genotyping marker data were scored using GeneMarker v1.91 software (SoftGenetics, State College, PA).

2.2.4 Disease evaluation approaches

Dr. Kiersten Wise (Department of Botany and Plant Pathology, Purdue University) provided four *F. graminearum* isolates: FG1, FG2, and FG2-23 collected from undesignated locations in Indiana in 2009, and isolate 10INSWF P5-2 collected from Vincennes, IN in 2010. The inoculum was prepared one month before use. Mycelia from the four isolates of *F. graminearum* were mixed and cultured in mung bean medium (Desjardins et al., 1996) with shaking at 200 rpm for 5-7 days at 23 to 25℃. The number of conidia was quantified using a hemocytometer and the inoculum was stored at 4℃ until use.

Type I resistance (FHB incidence (%); resistance to initial infection) was assessed on field-grown plants by scoring the percentage of infected spikes 14 days after spray inoculation at anthesis. Both sides of the spike were spray inoculated with a suspension containing 12,000 *F. graminearum* conidia spores per ml H_2O, either early in the morning or in the evening of the day on which 50% of the flowers were in anthesis. Data for FHB incidence was collected only at Lafayette, IN in 2013, since the evaluations of FHB incidence at Vincennes, IN were confounded by the presence of Stagonospora glume blotch. The FHB evaluation in 2012 at Lafayette, IN was affected by spring frost damage and drought conditions, so the plants were not scored.

Type II resistance (FHB severity (%): the spread of disease within the head) was assessed by scoring the percentage of infected spikelets within a spike 20-22 days after inoculation at anthesis in field-grown plants. At anthesis, the third spikelet from the tip of the spike was inoculated by injecting 10 µl of a suspension containing 50,000 conidia spores per 1 ml H_2O. A plastic bag covered the inoculated spike for 3 days to maintain

humidity. As with FHB incidence, FHB severity data were collected only at Lafayette, IN in 2012 and 2013 due to confounding Stagonospora glume blotch infection at Vincennes, IN.

The field misting system sprayed for 5 minutes every hour during daylight and ran for 2 weeks before most of the plants flowered and 1 week after flowering. Plants were only evaluated in 2013 for both FHB incidence and FHB severity data. The overall performance of plants exhibiting type I and type II resistance was determined by the FHB index (%), which was calculated by multiplying FHB incidence (%) and FHB severity (%) of each replication in 2013 field, and averaged over two replications.

2.2.5 Statistical analysis

In order to study the effects of QTL, the plant lines were separated into groups based on presence and absence of the five markers. For type I FHB resistance, the lines were divided into two groups: None (no markers) and 2B (presence of both Xbarc200 and Xgwm210 for the QTL *GF* on 2B). For type II FHB resistance the lines were divided into four groups: None (no markers), 7E (both Xcfa2240 and Xbf145935 for QTL *Qfhs.pur-7EL* without the marker for *Fhb1*), 3B (Xumn10 for gene *Fhb1* without markers for *Qfhs.pur-7EL*), and 7E+3B (Xumn10, Xcfa2240, and Xbf145935 for both QTL). Reciprocal transformation was applied to achieve the ANOVA requirement of normally distributed errors for FHB type II severity data in 2012 and 2013. For assessing FHB index (combinations of type I and type II resistance), eight groups were studied: None (no markers), 2B, 7E, 3B, 2B+7E, 2B+3B, 3B+7E, and 2B+7E+3B. One-way analysis of variance (ANOVA) was applied in the SAS ‘PROC GLM’ procedure, and mean of disease resistance (FHB incidence, FHB severity, and FHB index) of each QTL group

was estimated. Differences between group means was considered significant at a *p*-value of 0.05. The individual contributions of Truman, Bess, 99751 and INW0412 could not be evaluated since these lines had no markers associated with the FHB resistance that they contributed.

2.3 Results

2.3.1 FHB evaluations

Type I resistance was measured by scoring the percentage of infected spikes after inoculation. In the F_4 population, 59% of the lines showed lower FHB incidence than the population mean of 20.87% in 2013 over two replicated field tests (Fig. 2.1). The three checks INW0412, Bess, and 99751 all showed higher type I resistance than the population mean and 10% of the lines (24 lines) in the population had lower incidence of initial infection than those resistant checks (Table 2.3). Patterson, the FHB susceptible check, was highly susceptible with 52% of the spikes infected. Only five lines in the population had higher FHB incidence than Patterson in 2013. More than 85% of the lines had at least one marker associated with the major type I QTL from Goldfield, and at least 50% of the lines had both markers associated with the type I QTL *GF* from Goldfield.

Type II resistance was assessed in the F_3 and F_4 population by scoring the percentage of infected spikelets to which the disease had spread within a spike. FHB severity data were collected in two replicated trials in two consecutive years, 2012 and 2013. Since low humidity during the infection time in 2012 considerably reduced the chances of plants becoming infected by the pathogen, the mean disease severity for 2013 (8.56%) was almost twice as high as in 2012 (4.85%) (Table 2.3). Although INW0412 is a moderately resistant cultivar, in 2012 its FHB severity rating was 3.32%, which was

more resistant than the mean (4.85%) of plants in the study. However, in 2013 INW0412 received an FHB severity rating of 10.2% (Table 2.3), which was less resistant than the mean (8.56%). In 2012 and 2013, 22% and 79% of the lines, respectively, exhibited higher type II FHB resistance than INW0412. Under high FHB infection in 2013, the FHB severity distribution was skewed to resistance, and QTL *Fhb1* and *Qfhs.pur-7EL* played an important role in improving FHB resistance since their markers are present in the most resistant 92 lines (Figure 2.3, bar on the left).

The overall FHB resistance rating for 2013 was determined by calculating FHB index. In this F_4 population 69% of the lines showed higher FHB resistance than the mean (2.06%) (Figure 2.4) and 36% had higher overall FHB resistance than the moderately resistant check, INW0412 (FHB index = 0.98%). All three checks INW0412, Bess, and 99751 showed higher FHB resistance than the population mean and Patterson was susceptible to FHB.

2.3.2 Pairwise comparisons between groups

Wheat lines were sorted into two groups based on the presence or absence of markers for Type I resistance QTL *GF* from the donor, Goldfield wheat. 111 lines were excluded from the analysis because they contained only one of the markers on chromosome 2B, probably due to recombination or missing marker data. Since no markers were available for Bess, Truman, 99751 or INW0412. Type I resistance from these lines 1) could not be tracked and 2) contributed to both QTL groups; thus their contributions to the resistance of these groups could not be quantified. No significant difference was observed between the two QTL groups for type I FHB resistance (Table 2.4).

Wheat lines were sorted into four groups based on the presence or absence of markers for type II resistance QTL from the donors, Sumai3 and *Lophopyrum*. 120 lines were excluded from the analysis because they contained only one of the markers for the QTL on chromosome 7E or missing marker data. The four QTL groups (None, 7E, 3B, and 7E+3B) were compared based on the transformed FHB severity data from 2012 and 2013. The comparison for 2012, the year with unfavorable conditions for FHB disease development, showed no significant differences between groups (Table 2.5). Therefore, neither single QTL (*Fhb1* or *Qfhs.pur-7EL*) nor the QTL combination significantly improved type II FHB resistance when disease spread was severely limited by the environment. However, significant differences in FHB severity between QTL groups were observed for 2013 (Table 2.5). The group lacking QTL for type II resistance was significantly more susceptible to spread of the disease within a spike than groups with one or two QTL. And the group with both QTL for type II resistance was the most resistant.

Wheat lines were sorted into eight groups, based on the presence or absence of all markers for type I and type II resistance QTL, in order to assess the FHB index (overall resistance; Table 2.6). Only 75 lines were included due to 1) missing marker data (from recombination), because 2) no lines lacked all markers for the three QTL (the “None” group) and 3) no lines contained exclusively the markers for the 7E QTL (the “7E” group). In addition, three other groups were eliminated because of low numbers; groups 3B (lines contained only the markers for QTL *Fhb1*), 2B+7E (lines contained solely the markers for QTL *GF* and *Qfhs.pur-7EL*), and 3B+7E (lines contained only the markers for QTL *Fhb1* and *Qfhs.pur-7EL*) had 2, 3 and 3 lines respectively. Therefore, only

groups 2B, 2B+3B, and 2B+3B+7E were compared. The group with one QTL was more susceptible and thus had a higher FHB index estimate than groups with markers for two or three QTL. The resistance of groups 2B+3B and 2B+3B+7E was significantly higher than group 2B, while no significant differences were observed between groups 2B+3B and 2B+3B+7E. The differences among those three groups were also compared via box plots (Fig 2.5) showing a trend that FHB index decreased with increased marker numbers.

2.4 Discussion

We studied the contributions of three major QTL, individually and in combination, to FHB resistance. As a quantitative trait, the resistance to FHB is complex and the ability to detect the QTL is affected by environment, experimental error, and population size (Asins, 2002). The assessment of type I resistance (initial infection) is more sensitive to evaluation time, inoculum concentration and inoculation time than is assessment of type II resistance (spread of the disease) (Bai and Shaner, 2004), increasing the difficulty of accurately scoring the QTL. We followed the inheritance of two SSR markers associated with a QTL on chromosome 2B for type I resistance, low FHB incidence and narrow flower opening, which were previously identified from cultivar Goldfield over six environments (Gilsinger et al. 2005). Nonetheless, the group comparison identified no significant difference between plants carrying or lacking the QTL. This apparent lack of improvement in resistance was probably due to the presence of unmarked QTL from other type I FHB resistant cultivars including INW0412, Truman, Bess, and 99751, which masked the contribution by the marker-linked QTL from Goldfield wheat on 2B. However, 24 wheat lines were identified, with lower FHB

incidence than the resistant checks (Bess, INW0412 and 99751), suggesting that those lines may involve multiple type I resistance sources. These results support our first hypothesis that combing multiple type I FHB resistance sources would decrease the incidence of initial infection.

The QTL *Qfhs.pur-7EL,* from alien chromosome E of tall wheatgrass *Lophopyrum,* explains a large amount of phenotypic variation in type II FHB resistance (Shen et al. 2004; 2007) as does another popular major QTL on chromosome 3B, *Fhb1,* originating from a Chinese landrace. In our study, no significant difference was observed between wheat lines in group 7E and group 3B, which is reasonable since both QTL explained large amounts of phenotypic variation for type II resistance in previous studies (Table 2.1; Shen et al. 2006; 2007; Waldron et al. 1999; Anderson et al. 2001). In addition, Shen et al. (2006) demonstrated that the combination of *Qfhs.pur-7EL* and *Fhb1* significantly increased the type II FHB resistance in their test. Our results in 2013 supported that finding the combination of *Fhb1* and *Qfhs.pur-7EL* inhibited the spread of disease within the spike under high FHB infection. Additive effects mainly existed between those two QTL. However, similar results were not observed in 2012 when the overall disease severity was lower than 2013 due to environmental conditions that were not suitable for pathogen infection. Accordingly, using cultivars with multiple FHB resistance QTL is a powerful strategy, especially under conditions that promote high disease infection. In addition, our 2013 observations showed that of the 92 lines with high type II FHB resistance, 31% of them had all three type II markers and 96% of them had at least one type II marker.

For FHB index, the lowest index was observed in the group 2B+3B+7E. However, the improvement of FHB index for overall FHB resistance in the combined 2B+3B+7E group was not as high as expected when compared to group 2B+3B. Previous results showed that additive effects predominantly existed between QTL on 3B and 7E. Accordingly, the interaction effects existed either between QTL on 2B and 3B or between QTL on 2B and 7E, which influenced the FHB resistance of the lines within the pyramided QTL groups. Previous studies demonstrated that epistatic interactions between QTL influence the performance of pyramided QTL (Miedaner et al. 2006; Shinada et al. 2014). Therefore, we accepted that pyramiding QTL associated with type I and type II FHB resistance provided more effective resistance, while the interaction effects prohibited the augmentation of the resistance.

High FHB resistance lines were successfully identified in the population; 27 lines in the group with all major QTL on 3B, 7E, and 2B had a mean FHB index of 1.10%. But 14 lines with all three marker-linked QTL had an index lower than the resistance donor INW0412 (index 0.98%) and six lines were lower than the index of donor Bess (index 0.65%). The most resistant line with all three QTL had an index of 0.22% which is the lowest FHB index in the population except only one line had lower FHB index of 0.19% but with missing marker data (cannot be involved for FHB index analysis). Thus we accept our second hypothesis that pyramiding multiple QTL associated with type I and type II resistance would provide more effective resistance than fewer QTL.

This study successfully pyramided multiple sources of type I and type II FHB resistance to produce highly resistant wheat lines for crop improvement. In addition, the combination of three major marker-linked QTL is a useful resource since the marked loci

can be advanced together to decrease time required for production of future cultivars. Nevertheless, QTL pyramiding is considerably influenced by the interactions between QTL. The existence of epistatic effects could lead to unexpected results for QTL pyramiding. Moreover, the background of the donor and recipient lines should be well studied, since either unknown positive QTL or negative QTL would increase the complexity of the evaluation and affect the performance of cultivars in the field. Finally, the durability of resistance must be confirmed in multiple environmental tests.

Table 2.1 Donor parents and description of markers used for analysis

Donor Parents	QTL name	Chr.[1]	Markers	Distance covered (cM)	Phenotypic variation (%)	Reference
Type I Resistance, incidence or initial infection						
Goldfield	*GF*	2BS	Xgwm210, Xbarc200	35[2]	29	Gilsinger et al. (2005)
99751RA1-6-3-94	-	-	-	-	-	Ohm, 2011
Bess	-	-	-	-	-	Mckendry et al. 2007
Truman	-	-	-	-	-	Mckendry et al. 2005
INW0412	-	-	-	-	-	Unpublished data, Ohm
Type II Resistance, severity or spread within the head						
Sumai3	*Fhb1*	3BS	Xumn10	<4[3]	15.4-41.6	Waldron et al. (1999)
Lophopyrum	*Qfhs.pur-7EL*	7EL	Xcfa2240, Xbf145935	15[2]	15.1-32.5	Shen et al. (2007)
INW0412	-	-	-	-	-	Unpublished data, Ohm

[1] Chromosome

[2] Genetic distance between the two markers flanking the resistance QTL

[3] Genetic distance between one marker and the resistance QTL

Table 2.2 Primer sequences for wheat SSR and STS markers

QTL	Marker	Forward primer sequence	Reverse primer sequence	Reference
Fhb1	Xumn10	5'-CGTGGTTCCACGTCTTCTTA-3'	5'-TGAAGTTCATGCCACGCATA-3'	Liu et al. 2008
Qfhs.pur-7EL	Xcfa2240	5'-TGCAGCATGCATTTTAGCTT-3'	5'-TGCCGCACTTATTTGTTCAC-3'	Sourdille et al. 2001
	Xbf145935	5'-CTTCACCTCCAAGGAGTTCCAC-3'	5'-GCGTACCTGATCACCACCTTGAAGG-3'	Ayala-Navarrete et al. 2007
GF	Xbarc200	5'-GCGATATGATTTGGAGCTGATTG-3'	5'-GCGATGACGTTAGATGCGGAATTGT-3'	Song et al. 2005
	Xgwm210	5'-TGCATCAAGAATAGTGTGGAAG-3'	5'-TGAGAGGAAGGCTCACACCT-3'	Röder et al. 1998

Table 2.3 Phenotypic data summary

Lines	FHB incidence[1] (%)	FHB severity[2] (%)		FHB index[3] (%)
	2013	2012	2013	2013
INW0412	10	3.32	10.2	0.98
99751	9.83	7.16	16.96	1.61
Bess	9.83	6.57	7.34	0.65
Patterson	51.67	10.32	54.07	28.11
Lines Ave	20.87	4.85	8.56	2.06
Lines Max	62.5	27.38	75	46.25
Lines Min	5	1.79	3.87	0.19

[1] FHB incidence (%) was averaged over two replications in 2013

[2] FHB severity (%) was averaged over two replications in 2012 and 2013 individually

[3] FHB index (%) was calculated as following: {[(FHB incidence of replication 1 in 2013 × FHB severity of replication 1 in 2013) + (FHB incidence of replication 2 in 2013 × FHB severity of replication 2 in 2013)] / 2} ×100

Table 2.4 Quantitative trait loci combination groups for Fusarium head blight incidence in 2013

QTL Group	QTL-linked markers		Mean of FHB incidence estimate	n[1]	Significance[2]
	Xgwm210	Xbarc200			
2B	+	+	21.18	119	A
None	-	-	19.06	8	A

[1] Number of RILs with the indicated QTL-linked marker combination

[2] Only QTL groups with different letters are significantly different from each other, at p=0.05

Table 2.5 Quantitative trait loci combination groups for Fusarium head blight severity in 2012, 2013

Year	QTL Group	QTL-linked markers			FHB severity	Transformed Estimate[1]	n[2]	Significance[3]
		Xumn10	Xcfa2240	Xbf145935				
2012	7E+3B	+	+	+	5.33	0.2398	38	A
	3B	+	-	-	4.78	0.2470	45	A
	7E	-	+	+	4.93	0.2394	4	A
	None	-	-	-	4.90	0.2255	30	A
2013	7E+3B	+	+	+	5.71	0.1884	39	A
	3B	+	-	-	7.09	0.1561	45	AB
	7E	-	+	+	7.38	0.1460	4	B
	None	-	-	-	13.36	0.0923	30	C

[1] Transformed Estimate=mean of 1/FHB severity

[2] Number of RILs with the indicated QTL-linked marker combination

[3] Only QTL groups with different letters are significantly different from each other at p=0.05, based on the transformed data

Table 2.6 Quantitative trait loci combination groups for Fusarium head blight index in 2013

QTL Group	QTL-linked markers					FHB index	n[1]	Significance[2]
	Xgwm210	Xbarc200	Xumn10	Xcfa2240	Xbf145935			
2B	+	+	-	-	-	4.17	17	A
2B+3B	+	+	+	-	-	1.45	31	B
2B+3B+7E	+	+	+	+	+	1.10	27	B

[1] Number of RILs with the indicated QTL-linked marker combination

[2] Only QTL groups with different letters are significantly different from each other at p=0.05, based on the transformed data

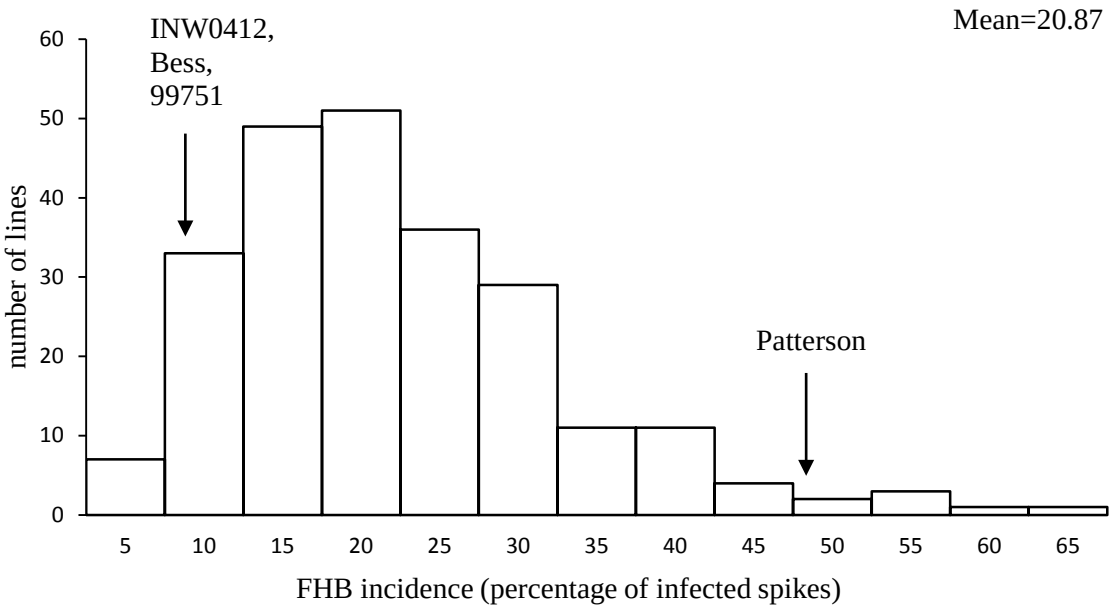

Figure 2.1 Frequency distribution of Fusarium head blight incidence in wheat F_4 population

Data were averaged over two replications in the field in 2013

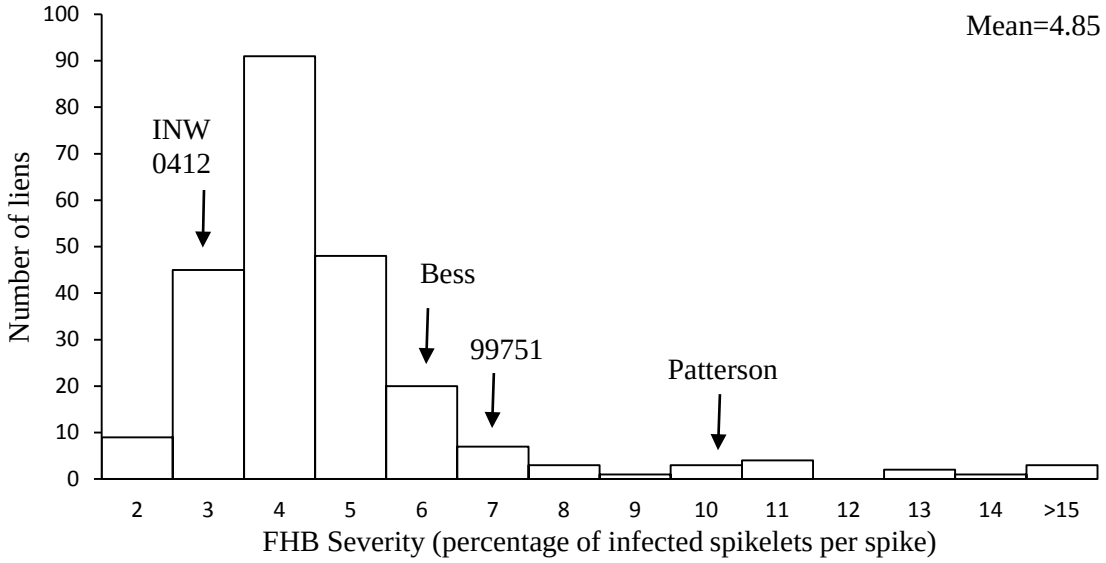

Figure 2.2 Frequency distribution of Fusarium head blight severity in the wheat F_3 population of 2012

Data were averaged over two replications in the field in 2012

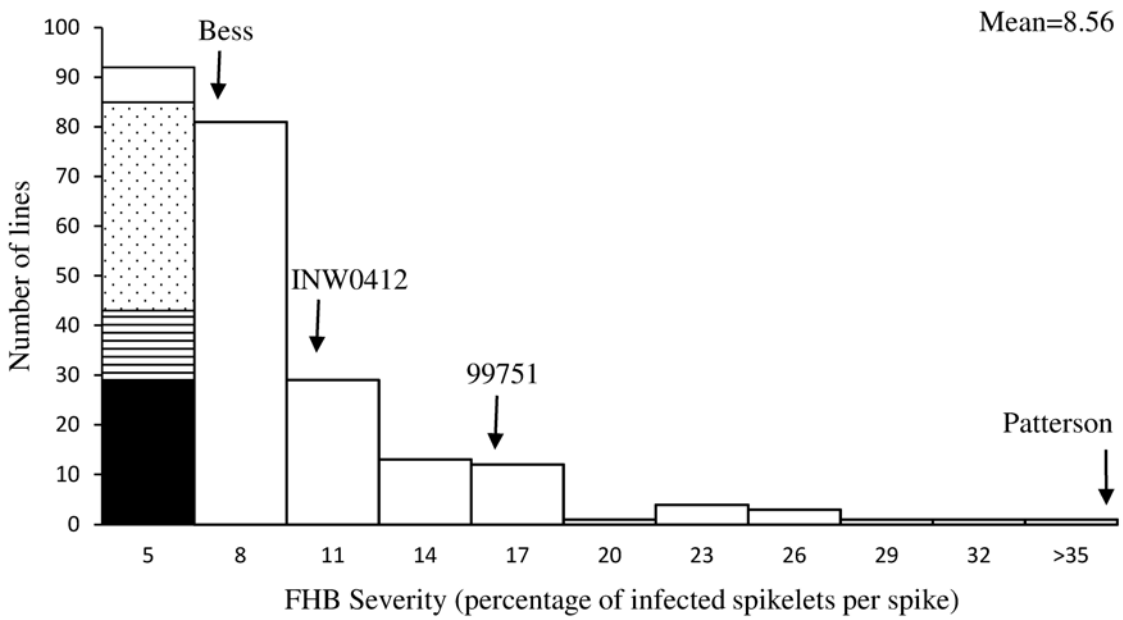

Figure 2.3 Frequency distribution of Fusarium head blight severity in the wheat F_4 population of 2013

Data were averaged over two replications in the field in 2013. The first bar showing low FHB severity (data for 92 lines) is divided into four parts; black indicates lines (29) with all three markers (Xumn10, Xcfa2240, and Xbf145935); stripes indicate lines (14) with two markers (Xumn10 and one of the markers on 7E); dots indicate lines (42) with only the Xumn10 marker; and white indicates the remaining lines including those with missing marker data (1), with both markers on 7E (2), with only oKine marker on 7E (1), or with no markers (3).

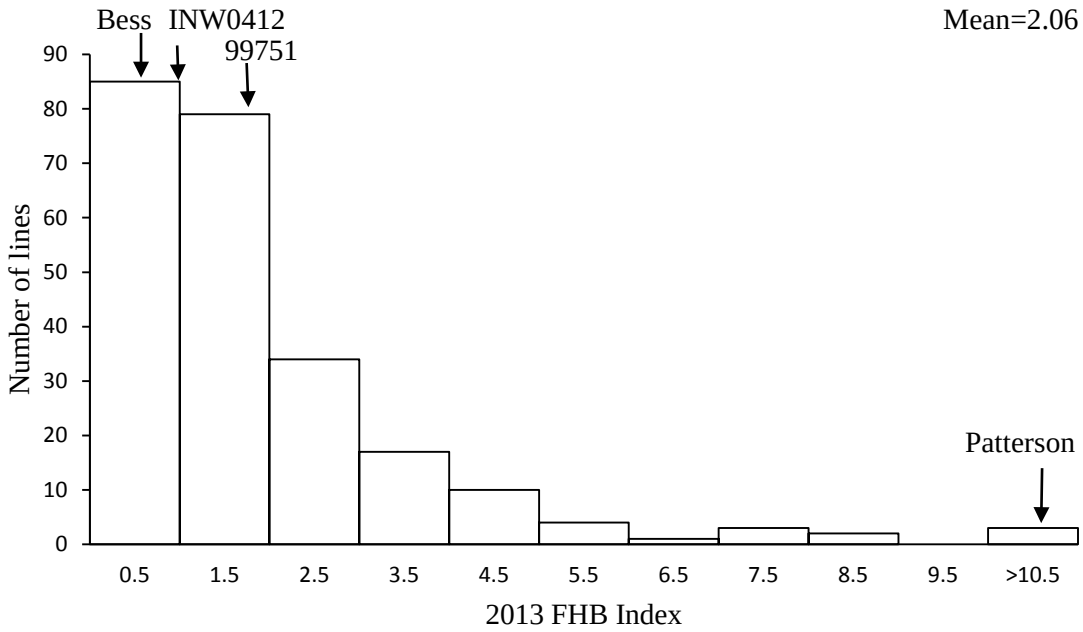

Figure 2.4 Frequency distribution of Fusarium head blight index in the wheat population in the field in 2013

FHB index (%) of 2013 was calculated as following: {[(FHB incidence of replication 1 in 2013 × FHB severity of replication 1 in 2013) + (FHB incidence of replication 2 in 2013 × FHB severity of replication 2 in 2013)] / 2} ×100

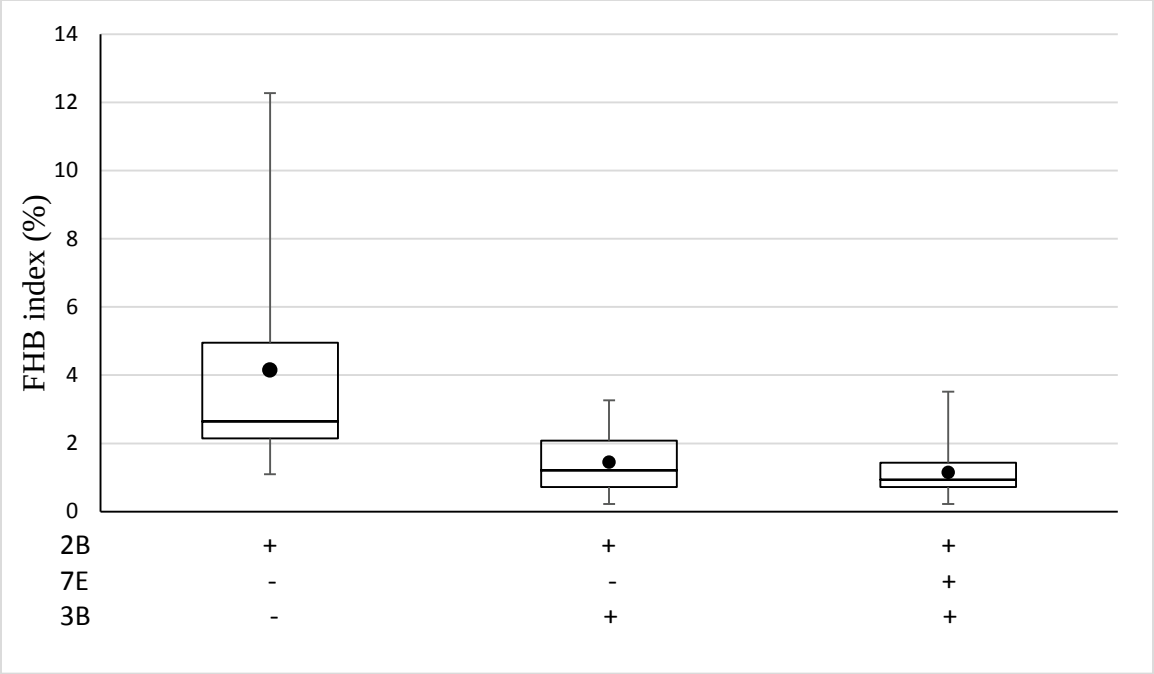

Figure 2.5 Association of Quantitative trait loci-linked markers with Fusarium head blight index

FHB index over three QTL groups, based on the presence of three markers (Xbarc200, Xumn10 and Xcfa2240) linked to three QTL on chromosomes 2B, 3B and 7E. The X-axis shows the QTL-linked markers, used to sort the RILs into three groups; + and – represent the presence and absence of specific markers. The Y-axis represents the FHB index (%). The boxes represent 75%, 50% (or median), 25% quantile from top edge to center line to bottom edge. The top and bottom bars represent maximum and minimum FHB index respectively, and the dots refer to the mean of FHB index. FHB index (%) was calculated as following: {[(FHB incidence of replication 1 in 2013 × FHB severity of replication 1 in 2013) + (FHB incidence of replication 2 in 2013 × FHB severity of replication 2 in 2013)] / 2} ×100

CHAPTER 3. MAPPING QTL ASSOCIATED WITH TYPE I FHB RESISTANCE IN WINTER WHEAT INW0412

3.1 Introduction

Fusarium head blight (FHB) causes economic losses in many countries around the world (Bai and Shaner, 2004) due to wheat yield reduction and mycotoxin accumulation in the grain, which is harmful to humans and livestock (Boenisch and Schäfer, 2011). In North America, FHB is mainly caused by the fungus *Fusarium graminearum* (Parry et al, 1995). Utilizing FHB-resistant cultivars is more effective and economical than other management strategies (Gilbert et al. 2000).

In 1963, Schroeder and Christensen reported two types of resistances to FHB in wheat: resistance to initial infection (type I resistance) and resistance to the spread of disease within the head (type II resistance). Quantitative trait loci (QTL) associated with type II FHB resistance have been widely identified in various wheat cultivars around the world. A major QTL for type II FHB resistance, *Fhb1* on chromosome 3BS, is considered to have the largest effect of any known FHB resistance gene (Bai et al. 2004). *Fhb1* has been mapped multiple times in different resistance sources from China, including ‘Sumai3’ (Waldron et al. 1999; Anderson et al. 2001), ‘Ning7840’ (Bai et al. 1999; Zhou et al. 2002), ‘Huapei57-2’ (Bourdoncle et al. 2003) and ‘Ning894037’ (Shen et al. 2003). In addition to *Fhb1* on chromosome 3BS, additional QTL controlling type II

FHB resistance have been identified repeatedly on chromosomes 2A (Waldron et al. 1999; Anderson et al. 2001; Steiner et al. 2004), 4B (Waldron et al. 1999; Anderson et al. 2001; Jia et al. 2005), 5A (Buerstmayr et al. 2002; Somers et al. 2003; Steiner et al. 2004), and 6BS (Anderson et al. 2001; Yang et al. 2003; Cuthbert et al. 2007; Buerstmayr et al. 2009).

Compared to type II resistance, the assessment of type I FHB resistance is more complex since it is influenced by the inoculum concentration, inoculation time and evaluation time (Bai and Shaner, 2004). Although not as common as type II FHB resistance, loci conferring type I FHB resistance have been identified in several cultivars as well, including 'Wangshuibai' (Lin et al. 2006), 'Frontana' (Steiner et al. 2004), 'Remus' (Steiner et al. 2004), 'Goldfield' (Gilsinger et al. 2005), plus in tetraploid durum wheat (Ruan et al. 2012), with resistance on chromosomes 1B, 2B, 2D, 4B, 5A, 6B, 7A and 7B (Buerstmayr et al. 2009). Nonetheless, as a quantitative trait significantly influenced by the environment, more research is essential to detect additional sources of stable and efficient QTL for type I resistance for cultivar development.

Next-generation sequencing technologies, especially genotyping-by-sequencing (GBS; Elshire et al. 2011), provide important opportunities for plant breeding. GBS is valuable for crops like wheat with large genomes and limited public resources, because it uses restriction enzymes to reduce the sequence complexity for discovery of single nucleotide polymorphisms (SNPs) (Deschamps et al. 2012; Crespo-Herrera et al. 2014). Poland et al. (2012) have successfully developed high-density genetic maps using a two-enzyme system for GBS in wheat and barley. Crespo-Herrera et al. (2014) applied GBS to map novel aphid-resistance QTL in a wheat recombinant inbred line (RIL) population.

Preliminary field data indicated that wheat line ‘INW0412’ contained both type I and type II FHB resistance (unpublished data, Herbert Ohm). Since type II resistance in the line Huapei 57-2 (parent of INW0412) had been investigated previously (Bourdoncle and Ohm, 2003), we constructed a RIL population for the purpose of identifying type I resistance QTL. The current study had two goals: 1) to identify new QTL influencing type I resistance to FHB in INW0412 wheat, 2) to develop a genetic linkage map defining the chromosomal locations of these QTL and providing molecular markers for marker-assisted selection. While approaching these goals we tested two hypotheses 1) RILs with multiple type I resistance QTL provide lower incidence of initial infection than lines with fewer QTL, and 2) type I and type II FHB resistance are contributed by different QTL.

3.2 Materials and Methods

3.2.1 Development of wheat recombinant inbred lines

Winter wheat line INW0412 is the donor parent of moderate type I FHB resistance, originally from the Chinese cultivar Huapei 57-2 (Bourdoncle and Ohm, 2003), while ‘992060G1’ is susceptible to FHB and is derived from the moderately susceptible line ‘Patterson’ (Bourdoncle and Ohm, 2003). 198 RIL were developed from the cross of INW0412 and 992060G1. Both the F_7 and F_8 generations of the 198 RILs were seeded in 1m single-row plots, with one row of each line in 2011 and two rows of each in 2013 at the Agronomy Center for Research and Education (ACRE), Purdue University, West Lafayette, IN. F_8 RILs were also planted in the 2012 fall greenhouse and 2013 spring greenhouse at Purdue University, with 10 plants seeded for each RIL to estimate type I resistance to FHB.

3.2.2 Fungal materials and culture conditions

Dr. Kiersten Wise (Department of Botany and Plant Pathology, Purdue University) provided four *F. graminearum* isolates: FG1, FG2, and FG2-23 collected from undesignated locations in Indiana in 2009, and 10INSWF P5-2 collected from Vincennes, IN in 2010. The inoculum was prepared one month before use. Mycelia from the four isolates were mixed and cultured in mung bean medium (Desjardins et al., 1996) with shaking at 200 rpm for 5-7 days at 23 to 25℃. The number of conidia was quantified using a hemacytometer and the inoculum was stored at 4℃ until use.

3.2.3 Disease evaluation

Type I resistance (resistance to initial FHB infection, FHB incidence) was determined by scoring the percentage of infected spikes 14 days after spray inoculation at anthesis. Each spike were sprayed with a suspension containing 12,000 *F. graminearum* conidia spores per ml dH_2O per side, either early in the morning or in the evening of the day on which 50% of the flowers were at anthesis. The field misting system sprayed for 5 minutes every hour from 7am to 8pm, and the misting system in the greenhouse sprayed for 3 minutes every hour from 7am to 8pm, beginning 2 weeks before most of the plants flowered until 1 week after flowering. FHB incidence data were collected for greenhouse and field. For the 2013 field, type I resistance scores were averaged over two rows for each RIL.

3.2.4 DNA isolation and library preparation

198 F_8 RILs and both parental lines were planted in plastic trays containing growing mix soil (Sunshine Redi-earth professional growing mixes, Sun Gro Horticulture, Agawam, MA) and placed in a cold room for three days to break dormancy

at 2℃ with 12-hour light. Trays were then moved back to the greenhouse. The leaf tissue was collected at the 2-leaf stage for each RIL and both parents, frozen immediately in liquid nitrogen and stored at -80℃. Wheat DNA was extracted from leaf tissues using the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol. Initial quantification of DNA samples was done on a NanoDrop100 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

DNA samples were prepared for GBS according to Poland et al. (2012). Briefly, GBS libraries were constructed using a two-enzyme *PstI-MspI* protocol and barcode adapters were applied. The 198 RIL plus two parental samples were pooled into three libraries, PCR-amplified, and each library was sequenced on a lane of an Illumina HiSeq 2000 (San Diego, CA).

3.2.5 SNP calling

The GBS SNP reads were processed using the default parameters of the Universal Network Enabled Analysis Kit (UNEAK) pipeline (Lu et al., 2013), which is for species lacking a reference genome. UNEAK is part of the program Trait Analysis by aSSociation, Evolution, and Linkage (TASSEL) 3.0 standalone (Bradbury et al., 2007). SNPs with more than 30% of the RIL population having missing data and with the parental reads having more than 5% heterozygosity were removed from the dataset. In addition, SNPs were removed if reads showed more than a 10-fold difference in the frequency of the two homozygous progeny genotypes, an indicator of high segregation distortion.

3.2.6 Linkage map construction and QTL analysis

Linkage groups and marker order for the 198 F_8 RIL population were constructed in JoinMap 4.0 software (Van Ooijen, 2006), based on a minimum logarithm of the odds (LOD) threshold value of 3.0. SNPs were mapped to linkage groups and assigned to wheat chromosomes by searching the wheat genome published by The International Wheat Genome Sequence Consortium (IWGSC) (Mayer et al. 2014; http://www.wheatgenome.org/) with the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990). The Kosambi map function (Kosambi, 1944) was utilized to calculate the genetic distance between SNP loci. SNP markers with high stress values (>5 or <-5) were removed from the dataset. The composite interval mapping (CIM) function of Windows QTL Cartographer V2.5 (Wang et al. 2012) identified FHB resistance QTL using the Standard Model, 5 control markers, 10.0-cM window size, forward regression method, and 2.0-cM walking speed. Utilization of the CIM approach decreases the background noise from the other QTL (Bernardo, 2010). A 1000-permutation test (Doerge and Churchill, 1996) estimated the threshold of the LOD score for significance of a QTL value at the p=0.05 level for all traits and all chromosomes. And markers under the 95% confidence interval peak closely linked to each QTL were determined based on a 1.5-LOD support interval (Dupuis and Siegmund, 1999). Multiple interval mapping analysis was utilized to study the interaction effects among QTL using Windows QTL Cartographer V2.5. The initial multiple interval mapping model selection method was “scan through QTL mapping result file” based on previous CIM QTL results using the default parameters.

3.2.7 Statistical analysis of FHB incidence

For some analyses, two years of FHB incidence data for greenhouse or field studies were averaged for each RIL. These averaged datasets were referred to as combined greenhouse or field data for QTL identification. Analysis of variance (ANOVA) was applied in SAS (SAS 9.3, SAS Institute, Cary, NC) in the "PROC GLM" procedure, and each variance component was estimated using "PROC VARCOMP". Broad sense heritability was calculated based on the following formula: $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2 + \sigma_{g\times e}^2 + \sigma^2)$, where σ_g^2 is the genetic variance component for RILs, σ_e^2 is the environmental variance for four different environments, $\sigma_{g\times e}^2$ is the interaction of RILs × environments, and σ^2 is the error. RILs were grouped according to which QTL LOD-peak markers they possessed, and the means of each group were calculated using combined field data and compared using the "MEANS" statement in the "PROC GLM" procedure.

3.3 Results

3.3.1 Type I FHB resistance

The RILs showed continuous distributions in FHB incidence (type I resistance) over two years in both field and greenhouse environments (Figure 3.1). The FHB incidence data for the field was normally distributed, whereas the data for the greenhouse was skewed toward resistance (Figure 3.1). In both greenhouse and field, transgressive segregation was detected for FHB incidence in this RIL population (Figure 3.1). The FHB incidence for the parental lines, the mean incidence of the RIL population, maximum and minimum of RILs in field and greenhouse are summarized in Table 3.1. The distribution of the greenhouse data (0-90% for combined greenhouse data) had a

wider range of FHB incidence than did the field data (15-60% for combined field data). ANOVA showed that genotypes, environments, and the interactions between genotypes and environments (G×E) significantly influenced the FHB incidence ($p<0.0001$; Table 3.2). Broad sense heritability of FHB resistance was estimated to be 0.11 in the RIL population, which was influenced by environments, genotypes by environments interactions, and errors.

3.3.2 QTL mapping

154,390 SNP sequences were initially identified from the GBS output by the UNEAK pipeline. After filtering to remove markers with high levels of missing data, with heterozygosity within a parental line or with segregation distortion, 828 SNP markers were identified. BLAST analysis assigned all except for 9% of the SNPs to predicted chromosomal locations (Table 3.3). JoinMap constructed 16 linkage groups from the markers. Comparing the BLAST assignments with the JoinMap results allowed us to assign linkage groups to wheat chromosomes; however, not all markers within a linkage group were placed in that location by BLAST. Six chromosomes, five of which were from the D genome, lacked enough markers to construct a map (Table 3.3). And mapping was unable to coalesce the two linkage groups that corresponded to chromosome 6A. The final map included 703 high quality SNP markers covering 1882.57 cM of the hexaploid wheat genome.

Type I FHB resistance QTL were estimated independently for greenhouse and field data using CIM. On chromosome 1AS we detected one QTL, to be known as *Qfhs.pur-1AS,* based on the 2012 greenhouse data and the combined greenhouse data; a corresponding smaller peak from the 2013 data did not reach the 95% confidence interval

cut-off for this QTL (Figure 3.2, Table 3.4). *Qfhs.pur-1AS* accounted for 12.20% of the phenotypic variation in the combined greenhouse data for 2012 and 2013 (LOD 6.01). The SNP closest to the LOD peak for this QTL was TP126266 at 83.30 cM, with eight other markers within the LOD 1.5 support interval (which approximates the 95% confidence interval for QTL location), based on the combined greenhouse data (Figure 3.2, Table 3.5).

Under field conditions, one QTL, *Qfhs.pur-1BL*, was detected on the long arm of chromosome 1B with the 2013 field data and with combined 2011 and 2013 field data (Figure 3.2. Table 3.4). This QTL accounted for 11.49% of the phenotypic variation in the combined data (LOD 7.34). *Qfhs.pur-1BL* Marker TP188538 at position 104.73 cM is the closest to the LOD peak for *Qfhs.pur-1BL* with 13 additional tightly linked markers within the LOD 1.5 support interval, based on the combined field data (Figure 3.2, Table 3.5).

Qfhs.pur-2BL, the type I FHB resistance QTL on chromosome 2BL, was identified in the 2013 field data in addition to the combined 2011 and 2013 field data (Figure 3.2). *Qfhs.pur-2BL* accounted for 11.74% of the phenotypic variation in the combined data (LOD 7.59) with marker TP97022 closest to the LOD peak for the QTL and three additional markers within the LOD 1.5 support interval, based on the combined field data (Table 3.4 and 3.5).

Qfhs.pur-3AS, the type I FHB resistance QTL on chromosome 3AS, was identified in the 2011 field data and the combined 2011 and 2013 field data (Figure 3.2). *Qfhs.pur-3AS* accounted for 8.51% of the phenotypic variation in the combined data (LOD 5.32) with marker TP228487 closest to the LOD peak and four other markers

within the LOD 1.5 support interval, based on the combined field data (Table 3.4 and 3.5). The 64 bp parental sequences, for each SNP that is tightly linked to one of the four QTL, are displayed in Table 3.5.

3.3.3 QTL effects in the field

Multiple interval mapping analysis, using only the three QTL identified in the combined field data (*Qfhs.pur-1BL, Qfhs.pur-2BL* and *Qfhs.pur-3AS*) demonstrated that additive effects existed among them. RILs with any one of the three QTL-linked markers, TP188538 (1BL), TP97022 (2BL), and TP228487 (3AS), had significantly increased type I resistance (15.00%, 13.13%, and 15.30% respectively) compared to RILs without any of the markers, each of them was responsible for a similar level of improvement in FHB resistance (Table 3.6). In addition, RILs with two of the three QTL-linked markers and RILs with all three QTL-linked markers had significantly improved FHB type I resistance in the field, 25.93 to 26.98% and 33.06% respectively, compared to RILs with none of the markers. These data also suggested that additive effects existed among the three QTL linked to the markers (Table 3.6, Improvement column). A clear trend was observed indicating that higher resistance was achieved in RILs with more QTL, indicated by the presence of their markers (Figure 3.3).

3.4 Discussion

Currently, fewer QTL have been identified for type I FHB resistance than for type II resistance. Since type I and type II resistance protect the plant during different stages of disease development, the availability of new sources for type I resistance will make possible the construction of cultivars with pyramided resistance loci capable of extending the durability of these traits.

We were able to detect four type I resistance QTL in the RIL population in this study. These QTL exhibited variation in resistance phenotype under greenhouse and field environments. A wider range of FHB incidence was observed in the greenhouse relative to the field environment, based on two years of data. Also, more lines with low FHB incidence were observed in the greenhouse than the field. As stated by Bai and Shaner (2004), the evaluation of type I FHB resistance is sensitive, with variation in assessment environments leading to different results (Kolb et al., 2001). In addition, both temperature and relative moisture significantly influence the production and distribution of conidia, as well as the infection process (Kolb et al., 2001). In our experiments, the greenhouse conditions were more consistent than the field environments. Therefore, significant environmental effects, and interaction effects between genotypes and environments, resulted in different QTL controlling type I FHB resistance in different environments.

Single FHB type I resistance QTL provide only limited resistance, so multiple QTL need to be combined into the same cultivar to provide stable and high level resistance across many environments,. Lin et al. (2006) demonstrated that the combination of two major type I resistance QTL decreased the percentage of infected spikes by 47.2% in their population. Similarly, Cativelli et al. (2013) showed that combining two major QTL in their population reduced the FHB severity by about 38.5%. Our experiments compared changes in type I resistance as an increasing number of QTL were found in the RILs. These results supported our Hypothesis 1, because lines with the three QTL that contribute to field resistance (on 1BL, 2BL and 3AS) were significantly more resistant than lines with one or none of the QTL.

The type I resistance donor parent of our RIL population, INW0412, may also be a good source for type II resistance. INW0412 was derived from Huapei 57-2, and our susceptible parent 992060G1 was derived from Patterson. A RIL population constructed by crossing type II resistant Huapei 57-2 to Patterson detected a major QTL on 3BS for type II resistance (Bourdoncle and Ohm (2003), which was also detected in many Chinese wheat cultivars (Waldron et al. 1999; Bai et al. 1999; Shen et al. 2003). In addition Huapei 57-2 has QTL controlling type II FHB resistance on chromosomes 3BL, 3AS, and 5BL. Since our study identified QTL associated with type I FHB resistance on chromosomes 1AS, 1BL, 2BL, and 3AS, all known type I and II resistance QTL in these related lines are independent except for those on 3AS. Thus as stated by Miedaner (2003) and proposed in our first hypothesis, type I and type II FHB resistance appear to be controlled by different loci in wheat.

The GBS technique provided high-quality, sequence-based markers associated with each of our type I resistance QTL, thus fulfilling Goal 2. Maps of the four regions yielded between four and 14 tightly linked 64-base sequences within the LOD 1.5 support interval for each of the four type I resistance QTL. This assortment of QTL-linked sequences can be used by breeders to introgress the QTL into a variety of cultivars. By identifying sequence variation between INW0412 and the recipient cultivar anywhere within the 64-base sequence of one of the many QTL-linked markers, a breeder could design PCR-based KASP markers (LGC Genomics, Beverly, MA) for use in high-throughput genotyping.

In addition to mapping the four QTL, we mapped SNP markers throughout the wheat genome. However, a smaller proportion of polymorphic SNPs was observed on the

wheat D genome (7%) compared to the A (42%) and B (51%) genomes. Similar results were observed for the D genome in previous studies (Chao et al. 2009; Berkman et al. 2013), and may be attributed to the evolutionary history of hexaploid wheat. Chao et al. (2009) and Berkman et al (2013) both suggest that early and continuous gene flow between hexaploid *Triticum aestivum* (AuAuBBDD) and tetroploid *T. turgidum* (AuAuBB) contributed to increased genetic diversity within the A and B genomes, whereas limited gene flow occurred between the hexaploid *T. aestivum* and *Aegilops tauschii* (DD). Therefore, some QTL controlling type I FHB resistance on the D genome may not have been detected in our RIL population due to fewer SNPs. Although we originally identified 154,390 SNPs, 99.55% of them were eliminated due to more than 30% of the RIL population having missing data, parental reads having more than 5% heterozygosity, or more than 10-fold segregation distortion. Consequently, the genome coverage was not high and additional type I FHB resistance QTL may have gone undetected in our study.

INW0412 is a cultivar adapted to a wide area in the US with outstanding yield under tough conditions, including late planted, surviving wet, cold, coupled with good resistance to FHB and other important diseases (Redinbaugh et al. 2013). Therefore, it is an important cultivar for winter wheat breeding, especially for pyramiding QTL identified for type I and type II FHB resistance. Developing PCR-based molecular markers for those QTL will greatly enhance the application of marker-assisted selection and QTL pyramiding to create germplasms with durable resistance.

Table 3.1 Mean of Fusarium head blight incidence in resistance parent (INW0412), susceptible parent (992060G1), and RILs from the cross of above two parents

Lines	FHB incidence (%)					
	GH12[1]	GH13	GHCOM	FLD11	FLD13	FLDCOM
INW0412	7.14	0	3.57	-[2]	7.5	7.5
992060G1	91.67	62.5	77.08	-	55	55
RIL Mean	25.83	37.01	30.65	40.91	33.17	37.04
RIL Max	100	90	90	70	62.5	60
RIL Min	0	0	0	25	5	15

[1] GH12: Greenhouse 2012; GH13: Greenhouse 2013; GHCOM: Combined data of greenhouse 2012 and 2013. FLD11: Field data at Lafayette in 2011; FLD13: Field Lafayette 2013; FLDCOM: Combined field data of 2011 and 2013 at Lafayette.
[2] FHB field incidence data for parental lines were not available in 2011.

Table 3.2 Analysis of variance of 198 Recombinant Inbred Lines for Fusarium head blight incidence across four different environments[1]

Source	DF[2]	Mean Square	F Value	P Value
RIL genotypes (G)	197	409.58	4.16	<0.0001
Environments (E)	3	7609.05	77.21	<0.0001
RILs × Environments (G×E)	550	266.59	2.71	<0.0001
Error	198	98.55		

[1] Environments: two years of field data and two years of greenhouse data
[2] Degree of freedom

Table 3.3 Summary of map

Chr.[1]	Markers assigned[2]	Length (cM)	Markers mapped[3]
1A	53	181.61	51
1B	45	148.28	69
1D	13	-	-[4]
2A	38	94.21	35
2B	186	351.80	187
2D	14	-	-
3A	14	34.69	11
3B	33	77.52	20
3D	5	-	-
4A	26	86.18	27
4B	30	119.06	31
4D	9	-	-
5A	34	134.58	37
5B	30	92.80	31
5D	19	27.36	15
6A	43	68.88	41
6B	4	-	-
6D	16	-	-
7A	83	311.39	101
7B	9	73.48	10
7D	51	80.72	37
Unknown	73	-	-
Total	828	1882.57	703

Genome	Percentage of markers assigned	Length (cM)	Percentage of markers mapped
Genome A	35%	911.54	43%
Genome B	41%	862.94	50%
Genome D	15%	108.08	7%
Unknown	9%	-	-

[1] Chromosome inferred by the majority of BLAST hits for markers in each linkage group.
[2] Number of markers assigned to different chromosomes based on BLAST results
[3] Number of markers mapping to each linkage group using JoinMap, based on a minimum logarithm of the odds threshold value of 3.0. Some markers from other chromosome BLAST locations or from the unknown group mapped to linkage groups not predicted by BLAST, which resulted in a larger number of markers placed on the map than were assigned to each chromosome based on BLAST.
[4] Too few markers to construct a map

Table 3.4 Quantitative trait loci for type I Fusarium head blight resistance on chromosomes 1AS, 1BL, 2BL, and 3AS identified by composite interval mapping from the INW0412 and 992060G1 RIL population in the greenhouse and field

QTL	Test Data	PVE[1] (%)	LOD	LOD-Peak Marker	Peak (cM)	Interval[2] Markers	Interval Distance (cM)
Qfhs.pur-1AS	GH12	8.80	3.95	TP126266	83.30	TP239403-TP22769	76.25-87.45
	GH13	N[3]	N				
	GHCOM	12.20	6.01				
Qfhs.pur-1BL	FLD11	N	N	TP188538	104.73	TP53681-TP238991	94.02-114.68
	FLD13	8.36	5.50				
	FLDCOM	11.49	7.34				
Qfhs.pur-2BL	FLD11	N	N	TP97022	293.28	TP156090-TP110393	291.72-297.47
	FLD13	8.06	5.24				
	FLDCOM	11.74	7.59				
Qfhs.pur-3AS	FLD11	7.44	3.91	TP228487	28.58	TP235843-TP221448	22.03-34.69
	FLD13	N	N				
	FLDCOM	8.51	5.32				

[1] Phenotypic variance explained by the QTL
[2] Markers within the LOD 1.5 support interval
[3] None detected at significance level of p=0.05 using the 1000 permutation test

Table 3.5 Parental sequences (64bp) of SNP marker linked to genomic regions influencing type I Fusarium head blight resistance

QTL	Marker	Parental marker sequence (R/S[1])
Qfhs.pur-1AS	TP239403	TGCAGTGTATGCTATAAACAGTAGAAAGGGATAAAAGAATTAGCAGAAACAGTGTCACATAA**C**/**T**G
	TP45575	TGCAGCAAAACGGTAACAACAAACATCAAAACAAAA**T**/**C**AAAGCAAACAACCACCAAGAGAAACTA
	TP91879	TGCAGCCGCCTCCTCGTCATTCGCTATGGCCGTAGCCGTCTCC**G**/**A**ACGCCGATGACGGCGTCACC
	TP215063	TGCAGTAGAAGCC**A**/**G**TCACGACGTCAGACAACGCCACCACCCTACGCTTGTCCATCAACATACGC
	TP165741	TGCAGGCAAGGGCGCCAGGGCGGCCCACACAGGGTGGTAA**C**/**T**AGAGTTGAGGAGGGAATAGGGTT
	TP126266	TGCAGCT**G**/**A**TCCCACCTCCTCGCGGACCCAAAGCGGCGCCTTCAAGAAGGTGACGGAGCCAGGCA
	TP241540	TGCAGTTAACTTGAGAAACCTGC**G**/**C**TCTCTGACTTACTATGCATGATGAACTAATGACCAACCAG
	TP7351	TGCAGAAGAGGCTTTTGTGGGAAAAGGACTTTATCTTAAGAGGTTGTCTTACCA**T**/**C**GCCAAAGGG
	TP22769	TGCAGAGAGCGGCTAGCTGGGCGGCTGGGGGCTGCTAGCAAATAGTCCCAC**A**/**G**TCGCTAACCGAA
Qfhs.pur-1BL	TP53681	TGCAGCACAAAGTTCAGGAGCTTCTGCTTCTCGGTGTAGCGTCCGAACATG**G**/**A**TGTTGTCGCTGT
	TP105924	TGCAGCGCATGGCACAGATCTCCGTGGCAACTGCTATGCATGAATGGTGGGATAGAAGGGAG**T**/**G**C
	TP21685	TGCAGAGAAGTGACA**G**/**A**CTGAAACCATGCAAGGGGCTTGGGTGAGCAGAAGCACGTCCTCCTTGG
	TP64192	TGCAGCAGCCGAAGAAGCAGGAGCAGGCTCTCCTCGCTGAGGCCGACGAGCAGCCAGGCCTCC**T**/**C**
	TP88777	TGCAGCCCTGCCATTTCCGCTGCCTTCTTCTTCTCCACCAACTTTCTTTGTCGTCCAGTCAT**T**/**C**G
	TP6098	TGCAGAACTCGACTATGTAGCTCATCTTCTC**T**/**C**TTCTGCTGCTTGAACACGAGCGTGGAGTCATC
	TP14106	TGCAGACACGGTCCAGAATGGCGACGAAGTCCCTCGCCA**G**/**C**AATGAATATCCTCAGAGGGCGCGC
	TP188538	TGCAGGGGAACAAA**C**/**A**AAATTGTTTCAGATTGTAGACCGCTCGCGAATTGAATGCAGCACTGGTA
	TP188067	TGCAGGGCTGCCTCAGCTGAGAGCGGCCAGGGC**T**/**G**GCGAGGCTGAGGTGCAGCTCCTCCAAGGAC
	TP104668	TGCAGCGATTTACTGTGTCATGTACAGCCACCTAGCTGCCTCTTC**T**/**A**GTTTTCCTCTAATTGTAA
	TP148502	TGCAGCTTTTCATCGCATTCTTCACAGTCAAGAATCCTCTCCATGGTG**C**/**T**TGATCGCTTCCTTAC
	TP40009	TGCAGATGCTGAGATGTGTAGA**G**/**A**ACGGATGGGGGCGGCGAGGTTATGTAGGAAGCGGGAGGAAG
	TP29075	TGCAGAGGGGCCC**G**/**A**AGACCTCCATGAGAAGTCCGCGATTGGTACCACCTCCCTGCCGAGATCGG
	TP238991	TGCAGTGTAACAAAACAACCCATTCATACGTTATATTTCACGTCACAAATTT**A**/**C**TAGGTAGAAGT

[1] Resistant parent SNP (INW0412) / Susceptible parent SNP (992060G1)

Table 3.5 Continued

QTL	Marker	Parental marker sequence (R/S[1])
Qfhs.pur-2BL	TP156090	TGCAGGACTTTCTTGAGCCCATGATCGGCGTTTAT**C/T**TTGAACTGCGCACCTGCTAGTTCTAGGA
	TP97022	TGCAGCCTCTGAACTTGAGCCTAGCAATGAAAAATGTT**T/C**GACTGAATATTGAGGTAGACTTATT
	TP237814	TGCAGTGGGCTTGAAGATGGTTGCACCAAATCTAAGTGCA**G/A**GGATTGGTGTGCTGGCGTAGGTT
	TP110393	TGCAGCGCTCGCAGGGGGATTTGGTGTGTGAGATCC**C/A**TTTGGTGTGTATGAGAGAAGGAAGGGG
Qfhs.pur-3AS	TP235843	TGCAGTGGAGACAGTGCCTATTTGATTGTTCCAATTTCTAATATAAGTC**A/G**CCTGTTTCGGGTGT
	TP228487	TGCAGTCTGCTGATGGTGCCAAGCGTAGAACTGAAACTGGAACTGATGGAACG**G/A**TCGACGATGG
	TP159281	TGCAGGAGCTTCGAGCCGACCCCGCAGCATGTAGACCGCCGTCGCCCGTCCGCCGTCGGGAAT**C/T**
	TP11596	TGCAGAATGACCAAGCAAAGAAAGAGAAAATAACTTGACAAGG**A/G**TTAGGGGTTAGAGATATATC
	TP221448	TGCAGTCATCAAACTTGAAGACATGCTGTTTAAGGCCTTATTTG**T/C**ACCGCTTCTCTTTTTTGTC

Table 3.6 Quantitative trait loci combination groups for field Fusarium head blight incidence

QTL-linked markers			Mean of FHB incidence	n[1]	Significance[2]	Improvement[3] (%)
TP188538	TP97022	TP228487				
1B	2B	3A				
-	-	-	45.75	15	A	0
-	+	-	39.74	34	B	13.13
+	-	-	38.89	9	BC	15.00
-	-	+	38.75	13	BC	15.30
+	-	+	33.89	9	CD	25.93
-	+	+	33.44	8	CD	26.91
+	+	-	33.41	11	CD	26.98
+	+	+	30.63	16	D	33.06

[1] Number of RILs with the indicated QTL-linked marker combination

[2] Only QTL groups with different letters are significantly different from each other at p=0.05.

[3] Calculated as the following: (mean of FHB incidence with none of the markers - mean of FHB incidence in each QTL-linked marker combination) / mean of FHB incidence with none of the markers × 100

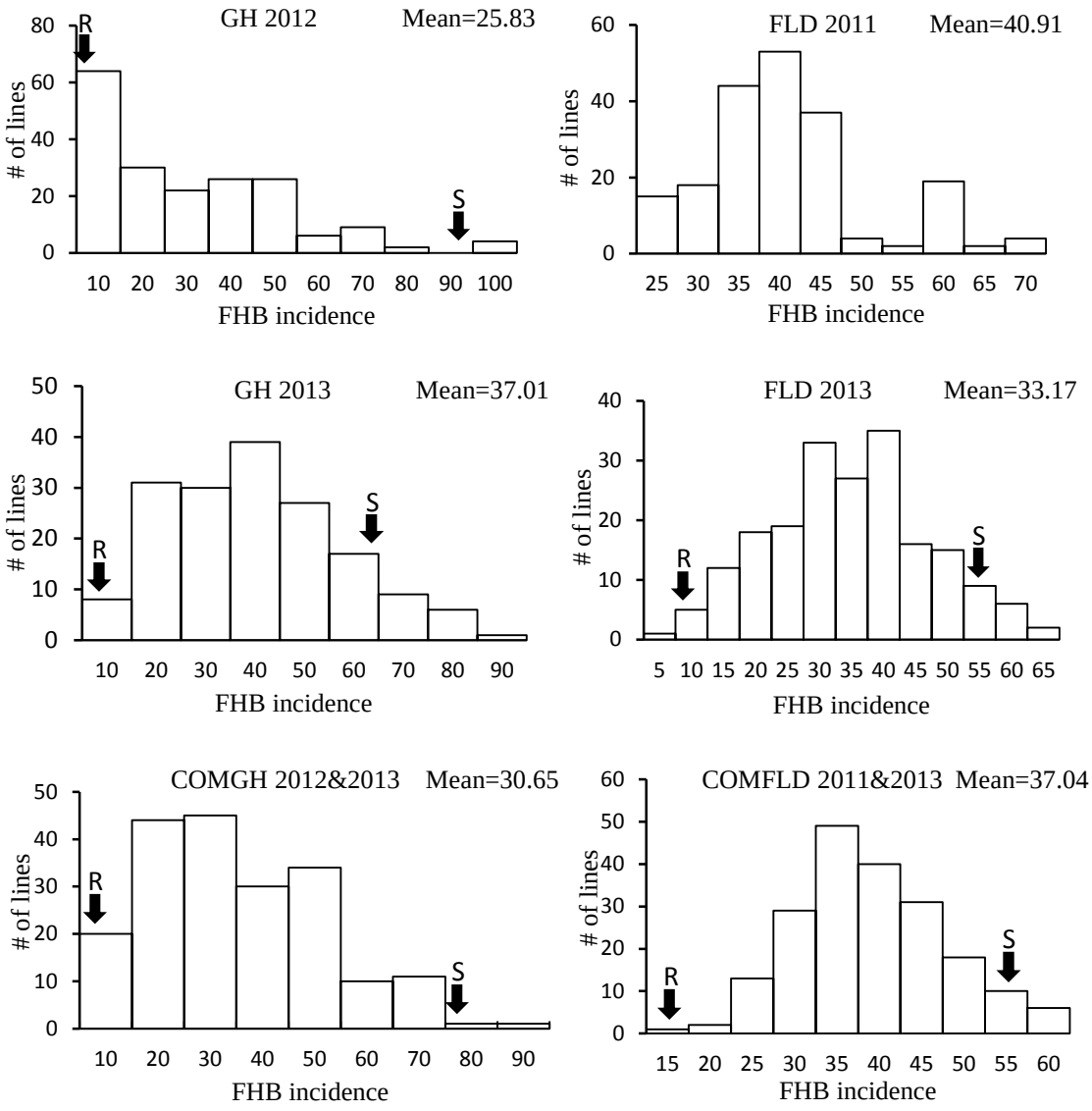

Figure 3.1 Fusarium head blight incidence distribution in the INW0412/992060G1 recombinant inbred lines

FHB incidence represents the percentage of infected spikes scored 14 days after spray inoculation. GH, Greenhouse; COMGH, Combined greenhouse data for both years; FLD, Field; COMFLD, Combined field data for both years; R, resistant parent INW0412; S, susceptible parent 992060G1. FHB incidence of resistant and susceptible parents for 2011 field is not available.

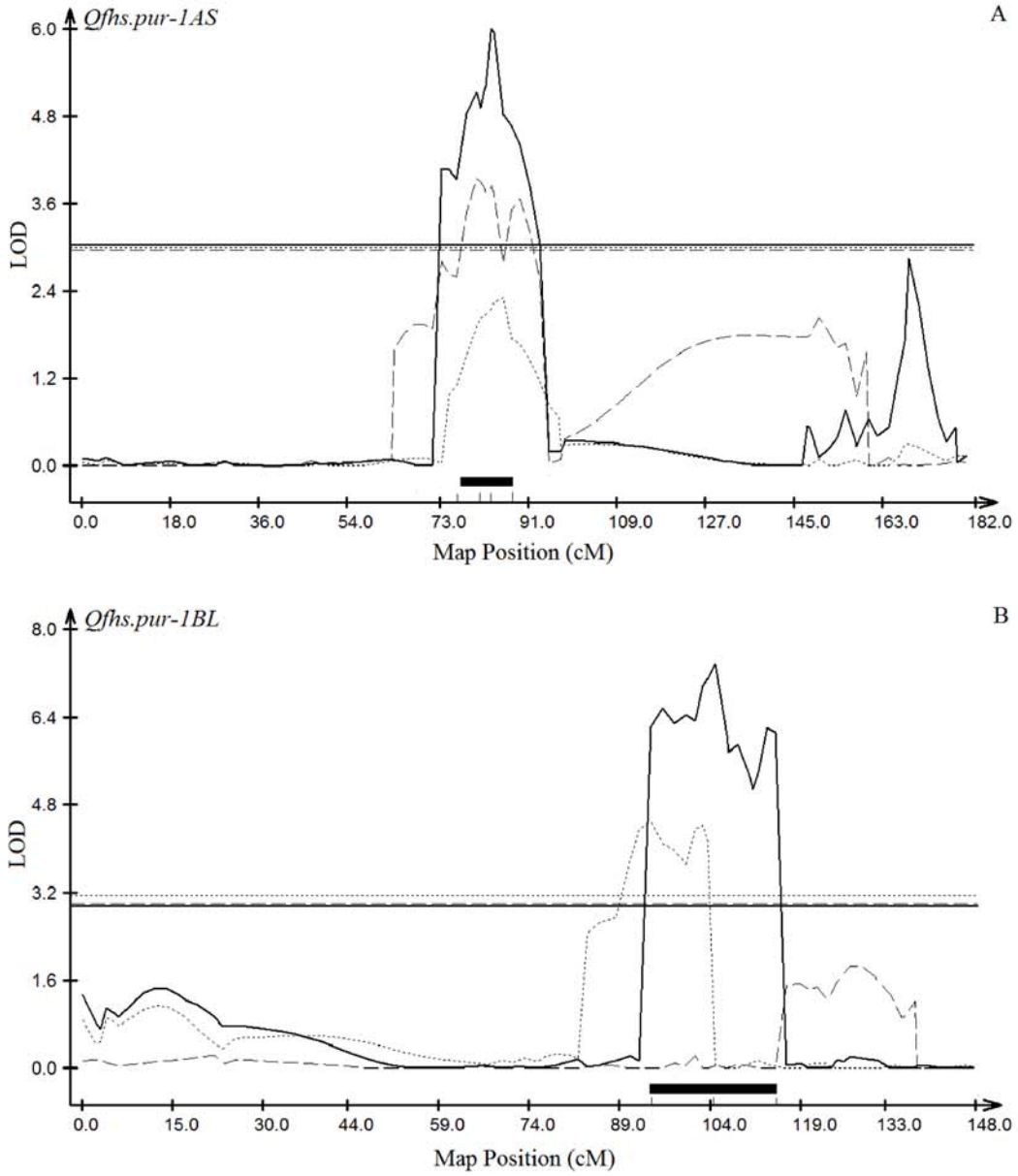

Figure 3.2 Quantitative trait loci influencing type I Fusarium head blight resistance

FHB incidence data from the 198 RIL population were used to detect QTL through composite interval mapping. Horizontal lines near the middle of each graph indicate the 95% confidence interval cut-off for the QTL calculated by the 1000 permutation test. The black bar represents the LOD 1.5 support interval for QTL-linked markers. Markers mapping to the LOD 1.5 support intervals for each QTL are listed in Table 3.5. A, The dashed line is for Greenhouse 2012 data; the dotted line is for Greenhouse 2013 data; the solid line is for combined data from greenhouse 2012 and 2013. B through D, The dashed line is for Field 2011 data; the dotted line is for Field 2013 data; the solid line is for combined data from Field 2011 and 2013.

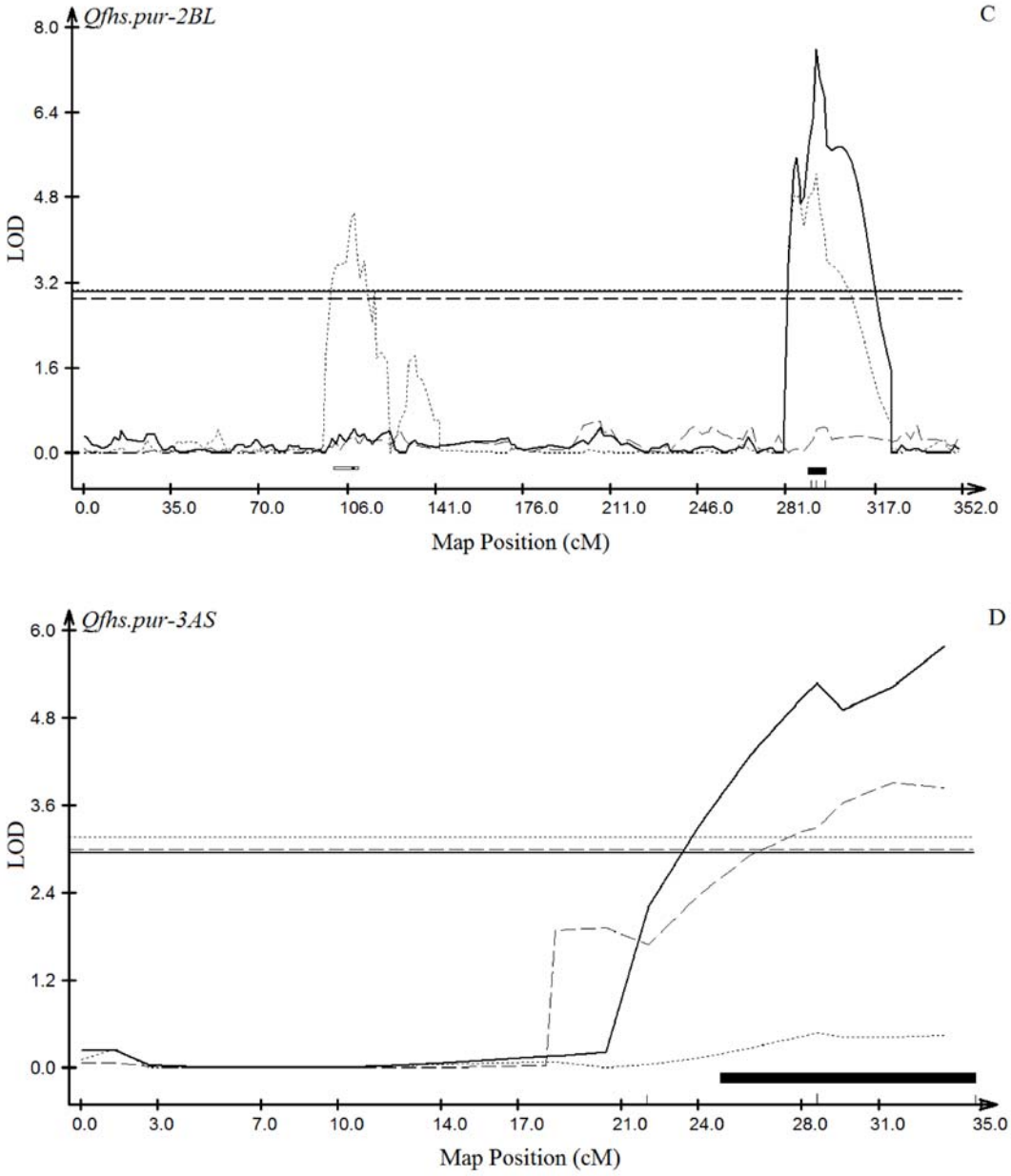

Figure 3.2 Continued

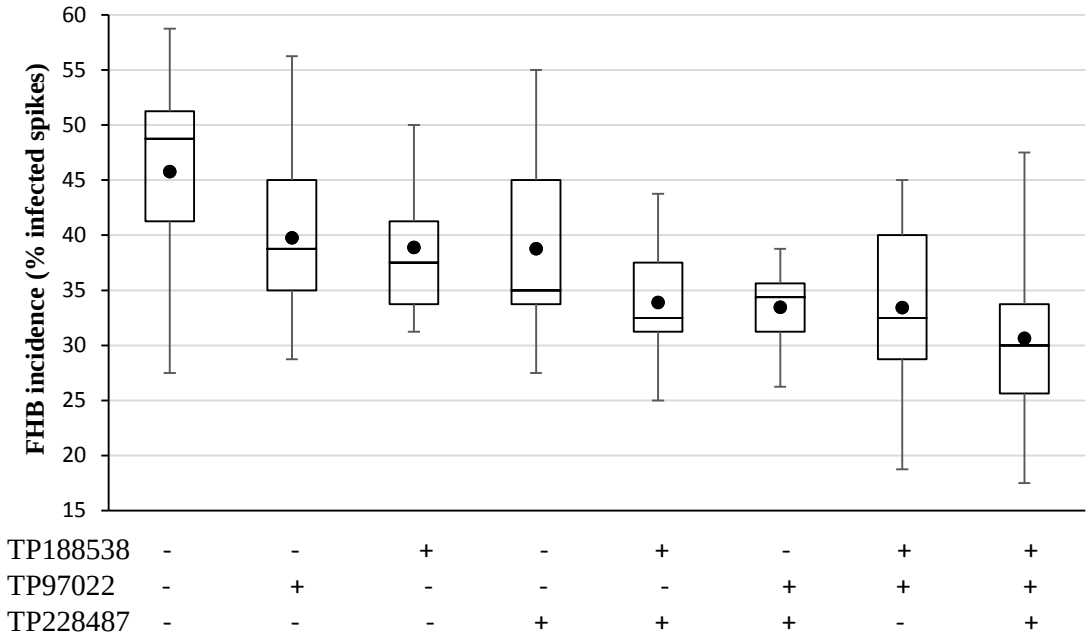

Figure 3.3 Association of Quantitative trait loci-linked markers with Fusarium head blight incidence in the field

FHB incidence over eight QTL groups, based on the presence of three markers (TP188538, TP97022, and TP228487) linked to three QTL on chromosomes 1B, 2B and 3A. The X-axis shows the QTL-linked markers, used to sort the RILs into eight groups; + and – represent the presence and absence of specific markers. The Y-axis represents the FHB incidence (% infected spikes). The boxes represent 75%, 50% (or median), 25% quantile from top edge to center line to bottom edge. The top and bottom bars represent maximum and minimum FHB incidence respectively, and the dots refer to the mean of FHB incidence.

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VITA

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